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DEVELOPMENT OF A STANDARD BIOMONITORING
METHODOLOGY FOR THE DETECTION OF TRACE
CONTAMINANTS WITHIN THE WATERS OF THE
ONTARIO GREAT LAKES REGION

R. A. C. PROJECT NO. 231C

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ACKNOWLEDGEMENT AND DISCLAIMER

ASSESSMENT OF CLAM (**ELLIPTIO COMPLANATUS**) STOCKS IN BALSAM LAKE

Introduction

Clams have been removed from Balsam Lake for use in biomonitoring projects mainly by the Ontario Ministry of the Environment (OME) and to a lesser extent by Environment Canada over the past six years. The clams have all been of the species **Elliptio complanatus**, which is by far the most abundant species in the lake. All the clams that have been removed have been from a small area of the lake at the village of Rosedale. During the period between 1980 and 1985 inclusive, approximately 300 to 1300 clams of the size range 6.5 cm to 7.2 cm were removed from the Rosedale Clam Bed per year for biomonitoring purposes. This report is an assessment of the current state of stocks of **E. complanatus** in Balsam Lake. The basic question to be answered is whether or not the Balsam Lake clam population will decline due to biomonitoring activity to the extent that other sources of clams would have to be sought. The Rosedale Clam Bed was investigated with regard to size distribution and total population. Size distribution was investigated, due to the difficulty of the more direct approach, age distribution. Age determination is difficult in the genus **Elliptio**. Unionid clam populations are vulnerable due to their very low recruitment rate. Loss in recruitment due to the removal of breeding adults was estimated. A search was also made for clam beds in other parts of the lake.

Methods

Size Distribution

The position of the Rosedale Clam Bed is shown with regard to Balsam Lake as a whole in Figure 1. Figure 2 shows the bed in more detail. The bed was defined as the area bounded on the south by the Trent Canal system channel, on the east by the shoreline of the lake, on the west by the depth at which clams could no longer be found and on the north somewhat arbitrarily as on Figure 2.

Two areas within the Rosedale Clam Bed were chosen for size distribution surveys. Site A chosen was off the north point, where we believe that very few clams have been collected. Site B was directly out from the dock of Balsam

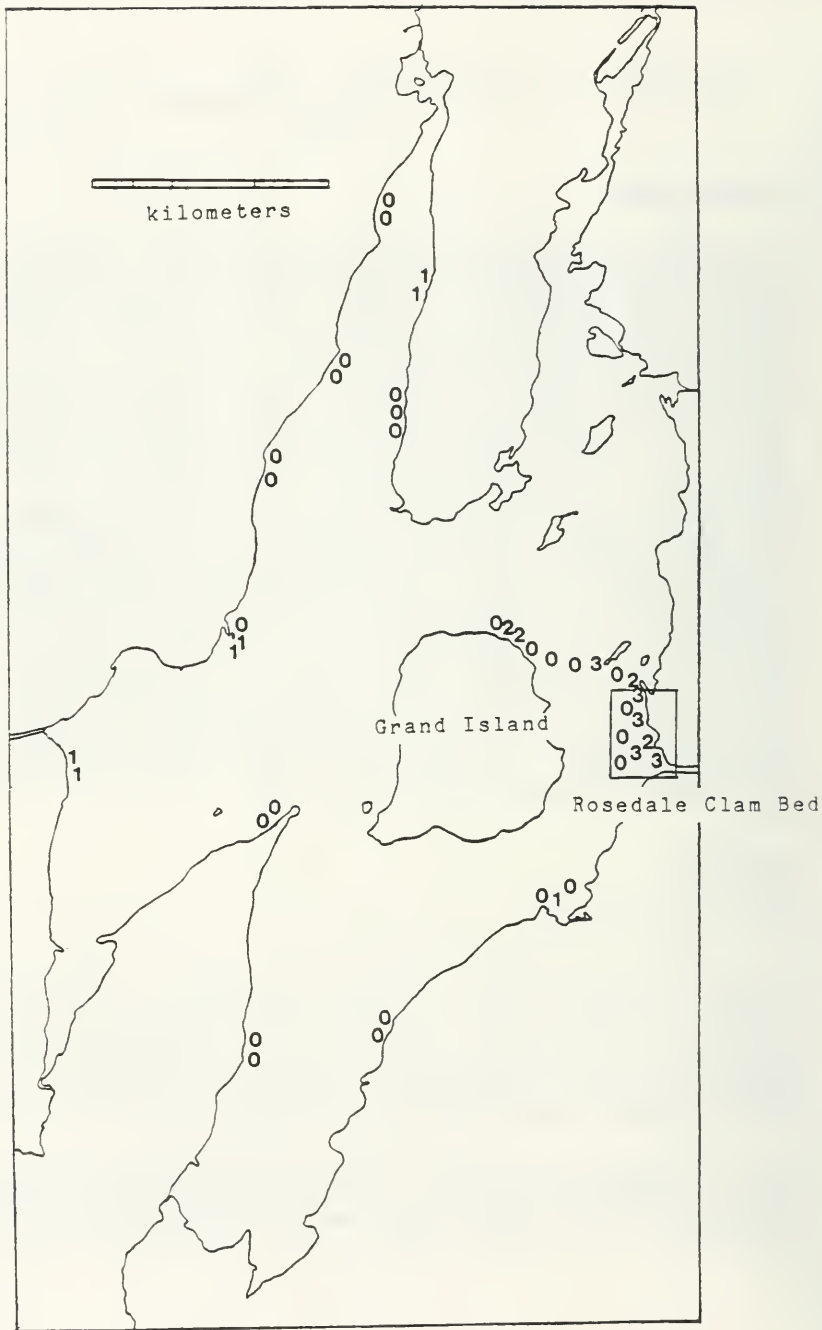Figure 1. Map of Balsam Lake showing areas where population
survey transects were run. Numbers refer to clam densities
(see Table 1). The area inclosed in the rectangle is the
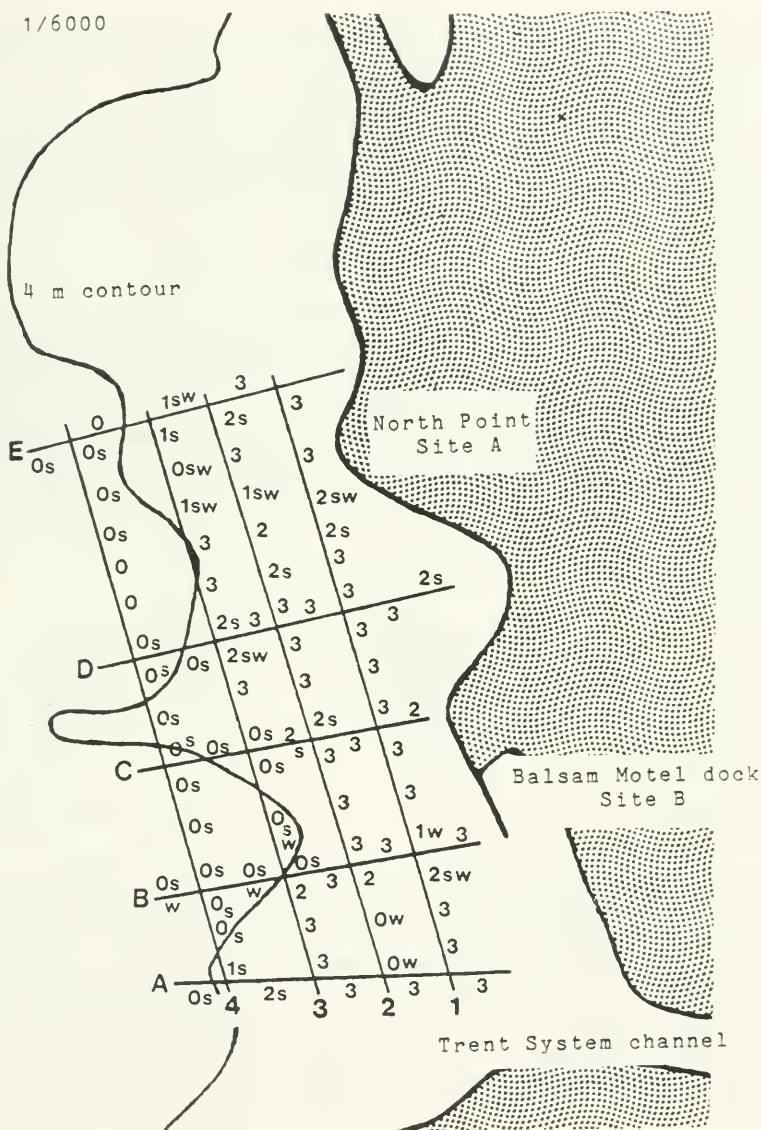Rosedale Clam Bed.

Figure 2. Map of the Rosedale Clam Bed area. The straight lines represent survey transects. Numbers are clam density codes (see Table 1). 's' indicates sand and/or silt and 'w' represents aquatic weeds.

Motel and Cottages. This area was chosen because it has been utilized extensively for supplying clams for biomonitoring purposes. The area has had many clams removed in the size range of 6.5 cm to 7.2 cm and perhaps some clams of other sizes as well. Both areas were similar in terms of substrate (small stones with sand in between) and depth (2 m). Within each site, random areas were selected. Within each area, all clams were collected by hand by SCUBA divers, brought to the surface and measured.

Population Survey of Rosedale Clam Bed

Observations of clams were made along transect lines, which are shown in Figure 2. Observations were made by a diver being towed by boat. A communications device enabled the diver's observations to be recorded by personnel in the boat on a map of the study area. Previous to this survey, counts of clams in several 1 m^2 areas enabled the diver to judge clam densities in a semi-quantitative manner. A clam Density Code was adopted as descriptive shorthand for the survey work. This code is described in Table 1.

Table 1. Clam Density Terminology

Description	Density Code	Mean
Abundant	3	$75/m^2$
Frequent	2	$15/m^2$
Scarce	1	$3/m^2$
Absent	0	$0/m^2$

Additional Sites for Clam Collection from Balsam Lake

During the survey of the Rosedale Clam Bed, it was found that **Elliptio complanatus** was approximately restricted to the depth range of 1 m to 4 m. It was also predominantly found in areas with a stony bottom. Other areas of Balsam Lake were surveyed by the same method as the Rosedale Clam Bed to determine the distribution of **E. complanatus** in the lake. Areas investigated were mostly confined to the 2 m to 3 m depth range in order to increase the probability of finding clams. However, one transect was made from the eastern shore to Grand Island. This transect took in depths up to 7 m.

Results and Discussion

Size Distribution

Although clams were abundant in both areas, we found that Site B had a lower clam density than Site A. We collected 755 clams from 5 m^2 in Site A (density: 151/m^2) and 436 clams from 7 m^2 in Site B (density: 62/m^2). The numbers, proportions and cumulative proportions of clams collected in each 1 mm size class are presented in Appendix A. Figure 3 shows the proportions and cumulative proportions.

The size distributions from both sites are typical of long-lived organisms with low recruitment rate. Characteristic is the prominant right skewness of the size frequency distribution. The proportion of clams in the 'collectable' size range (6.5 cm to 7.2 cm) is not greatly different at the two sites (28% at Site A and 32% at Site B). There is a lower proportion of smaller clams (under 6.5 cm) at Site B (54%) than at Site A (63%), but most of the difference can be accounted for in the 4.8 cm to 6.4 cm range. Recruitment does not seem to have suffered at Site B.

Population Survey of Rosedale Clam Bed

The results of this survey are shown in Figure 2. Within the Rosedale Clam Bed, it seems that clams are abundant everywhere where the substrate consists of small stones with sand between them. Population densities were lower where the bottom consisted entirely of sand or silt especially where there were weeds. Clam densities declined rather abruptly at a depth of about 4 m even where the substrate was favorable.

The mean densities corresponding to the density codes (Table 1) and the number of occurances of each density code within the clam bed were used to calculate a $\log$-mean clam density for the entire bed of approximately 23/m^2. From a navigation chart, the area of the clam bed as defined here was calculated to be about 16 ha. The total clam population of the bed is therefore about 3.7 million.

As shown in Table 2, this population suffers a direct loss of about 850/yr due to biomonitoring programs. From our own observations as participants in the biomontoring proram of the OME, we are sure that the clams harvested are all of breeding age. There is thus also a indirect loss due to
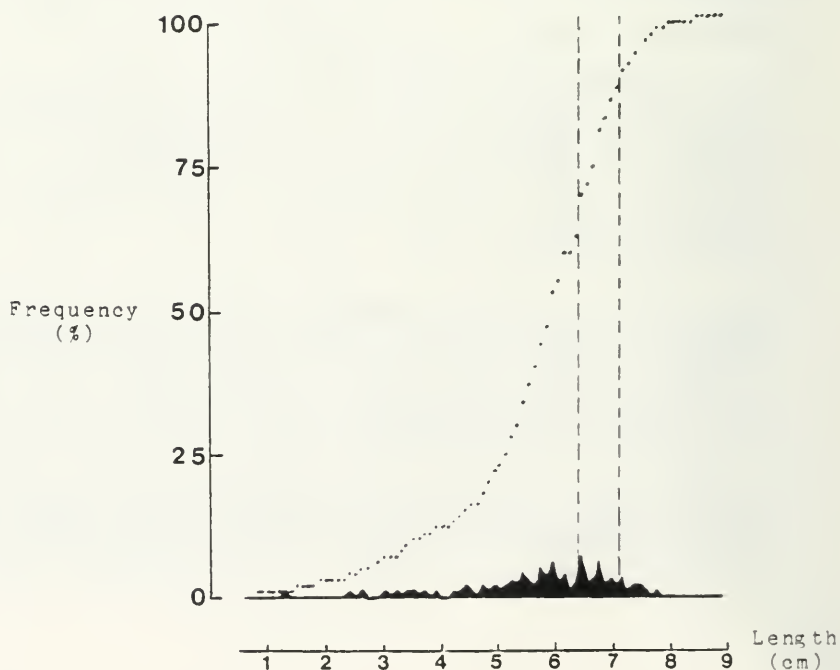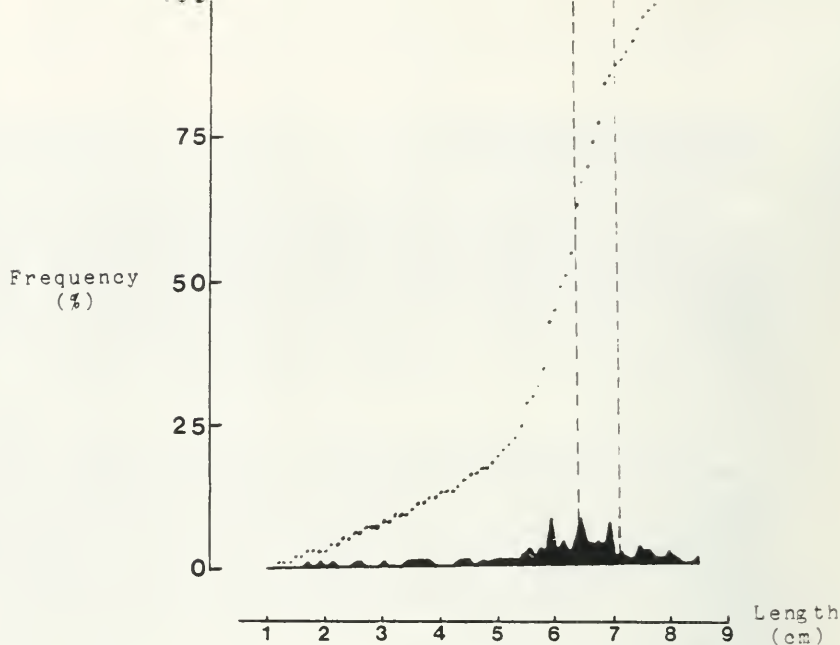
Figure 3. Comparison of the size frequency distribution (shaded area) and cumulative size frequency distribution (dotted curve) of two sites in the Rosedale Clam Bed. The upper graph represent Site A (unharvested) and the lower graph represent Site B (harvested). The area between the vertical dashed lines represents the size range of clams harvested for biomonitoring.

reduction of recruitment. The exact recruitment rate is not known, since the size of young of the year clams is not known precisely, although it probably lies in the range of 1.0 cm to 2.0 cm. The exact size of the breeding population is also not known, but is taken to include all clams of 6.5 cm or larger. The breeding population by this definition is about a third of the total population (see Appendix A) or 1.2 million. The average direct reduction in breeding population is therefore about 0.07%. One can assume that the reduction in annual recruitment is also 0.07%. This is certainly not a true assumption, since among breeding clams, larger individuals will produce more offspring than smaller individuals. However, the assumption is a conservative one in that it overestimates the effect of removal of breeding clams from the bed. If young of the year clams are 1.0 cm and under, the reduction in recruitment for the entire clam bed will be 8 clams per year. Even if the young of the year reach 2.0 cm, the reduction in recruitment will only be 74 clams per year.

Table 2. Approximate Numbers of Clams Removed from the Rosedale Clam Bed, Balsam Lake

Year	Clams removed
1980	825
1981	725
1982	275
1983	1050
1984	1350
1985	900
average	850

It can be seen from these calculations that in an average year the total expected population loss due to biomonitoring activities is not more than one thousand clams out of a total population of close to four million. This is an insignificant reduction. Clams compete with each other in their filter feeding activity and because of this their growth rates are higher when their population densities are lower. Larger size leads to increased reproductive output so that the population reduction is probably self-correcting.

Additional Sites for Clam Collection from Balsam Lake

The results of this survey are shown in Figure 1. Clams

were either scarce or absent on most survey transects. No
other clam beds comparable to the Rosedale Clam Bed in
population density or areal extent were found in other parts
of the lake. All clams observed were **Elliptio complanatus**.

Conclusions

1. The effect of harvesting clams for biomonitoring studies
from the clam bed at Rosedale on Balsam Lake is
insignificant.

2. Others parts of Balsam Lake should not be expected to
represent an alternate source of clams for biomonitoring,
since population densities are too sparse to permit easy
collecting.

Appendix A: Size Distribution of Clam Populations

Length (cm)	Clams Sampled from Site A:			Clams Sampled from Site B:		
	No.	Proportion (%)	Cumulative Proportion (%)	No.	Proportion (%)	Cumulative Proportion (%)
0.7	3	0.4	0.4	0	0.0	0.0
0.8	0	0.0	0.4	0	0.0	0.0
0.9	1	0.1	0.5	0	0.0	0.0
1.0	0	0.0	0.5	0	0.0	0.0
1.1	0	0.0	0.5	1	0.2	0.2
1.2	2	0.3	0.8	2	0.4	0.6
1.3	0	0.0	0.8	1	0.2	0.8
1.4	4	0.5	1.3	1	0.2	1.0
1.5	1	0.1	1.5	2	0.4	1.4
1.6	2	0.3	1.7	1	0.2	1.6
1.7	1	0.1	1.8	0	0.0	1.6
1.8	2	0.3	2.1	6	1.2	2.8
1.9	2	0.3	2.4	0	0.0	2.8
2.0	2	0.3	2.6	3	0.6	3.4
2.1	0	0.0	2.6	0	0.0	3.4
2.2	2	0.3	2.9	3	0.6	4.0
2.3	0	0.0	2.9	1	0.2	4.2
2.4	1	0.1	3.0	2	0.4	4.6
2.5	6	0.8	3.8	1	0.2	4.8
2.6	2	0.3	4.1	5	1.0	5.8
2.7	4	0.5	4.6	3	0.6	6.4
2.8	6	0.8	5.4	2	0.4	6.8
2.9	2	0.3	5.6	1	0.2	7.0
3.0	3	0.4	6.0	2	0.4	7.4
3.1	4	0.5	6.6	3	0.6	8.0
3.2	2	0.3	6.8	2	0.4	8.4
3.3	5	0.7	7.5	1	0.2	8.6
3.4	3	0.4	7.9	1	0.2	8.8
3.5	9	1.2	8.1	3	0.6	9.4
3.6	5	0.7	9.7	3	0.6	10.0
3.7	0	0.0	9.7	3	0.6	10.6
3.8	7	0.9	10.7	3	0.6	11.2
3.9	1	0.1	10.8	4	0.8	12.0
4.0	6	0.8	11.9	2	0.4	12.4
4.1	3	0.4	12.0	2	0.4	12.8
4.2	2	0.3	12.3	2	0.4	13.2
4.3	5	0.7	12.9	0	0.0	13.2
4.4	5	0.7	13.6	5	1.0	14.2

	Clams Sampled from Site A:			Clams Sampled from Site B:		
Length (cm)	No.	Proportion (%)	Cumulative Proportion (%)	No.	Proportion (%)	Cumulative Proportion (%)
4.5	13	1.7	15.3	4	0.8	15.0
4.6	6	0.8	16.1	5	1.0	15.9
4.7	3	0.4	16.5	2	0.4	16.3
4.8	15	2.0	18.5	4	0.8	17.1
4.9	8	1.1	19.5	0	0.0	17.1
5.0	17	2.3	21.8	4	0.8	17.9
5.1	10	1.3	23.1	4	0.8	18.7
5.2	17	2.3	25.4	7	1.4	20.1
5.3	19	2.5	27.9	6	1.2	21.3
5.4	13	1.7	29.6	5	1.0	22.3
5.5	33	4.4	34.0	11	2.2	24.5
5.6	25	3.3	37.3	16	3.2	27.6
5.7	18	2.4	39.6	6	1.2	28.8
5.8	35	4.6	44.3	13	2.6	31.4
5.9	21	2.8	47.1	11	2.2	33.6
6.0	42	5.6	52.6	42	8.3	41.9
6.1	18	2.4	55.0	12	2.4	44.3
6.2	30	4.0	59.0	19	3.8	48.1
6.3	11	1.5	60.4	12	2.4	50.4
6.4	18	2.4	62.8	18	3.6	54.0
6.5	53	7.0	69.8	40	7.9	62.0
6.6	17	2.3	72.1	21	4.2	66.1
6.7	25	3.3	75.4	15	3.0	69.1
6.8	45	6.0	81.4	22	4.4	73.5
6.9	16	2.1	83.5	13	2.9	76.1
7.0	19	2.5	86.0	35	6.9	83.0
7.1	14	1.9	87.9	6	1.2	84.2
7.2	25	3.3	91.2	8	1.6	85.8
7.3	7	0.9	92.1	5	1.0	86.8
7.4	12	1.6	93.7	5	1.0	87.8
7.5	18	2.4	96.1	13	2.6	90.3
7.6	6	0.8	96.9	10	2.0	92.3
7.7	2	0.3	97.1	9	1.8	94.1
7.8	8	1.1	98.2	5	1.0	95.1
7.9	0	0.0	98.2	3	0.6	95.7
8.0	3	0.4	98.6	11	2.2	97.9
8.1	1	0.1	98.7	3	0.6	98.5
8.2	1	0.1	98.8	2	0.4	98.9
8.3	3	0.4	99.2	0	0.0	98.9
8.4	0	0.0	99.2	1	0.2	99.1
8.5	3	0.4	99.6	5	1.0	100.1

Length (cm)	Clams Sampled from Site A:			Clams Sampled from Site B:		
	No.	Proportion (%)	Cumulative Proportion (%)	No.	Proportion (%)	Cumulative Proportion (%)
8.6	0	0.0	99.6	0	0.0	100.1
8.7	0	0.0	99.6	0	0.0	100.1
8.8	1	0.1	99.8	0	0.0	100.1
8.9	1	0.1	99.9	0	0.0	100.1

DEVELOPMENT OF A STANDARD CLAM BIOMONITORING METHODOLOGY
FOR THE DETECTION OF TRACE CONTAMINANTS
WITHIN WATERS OF THE ONTARIO GREAT LAKES REGION

Assessment of Available Clam Stocks

TASK A2 – ALTERNATE SOURCES

Prepared by Karen Edmunds and Al Melkic

INTRODUCTION

Freshwater clams have been used as biomonitoring agents by
the Ministry of the Environment for the past eight years
because of their ability to accumulate trace contaminants in
their tissues from the ambient environment. This methodology
has allowed detection of point source contamination in water
bodies where other conventional analytical methods had
previously been unsuccessful.

The clam species Elliptio complanata has traditionally been
harvested for biomonitoring purposes, from an area in Balsam
Lake in the Kawartha region, where a large, dense and
uncontaminated clam population exists. While this community
is an easily accessible and reliable source, it is limited to
primarily one species and the question arises as to the
accumulative capacity of other clam species. Other clam
species from environmentally different lakes or Elliptio from
a different source, may be more compatible in certain waters,
and therefore more sensitive as biomonitors.

In order to investigate the possibility of utilizing
alternate clam species and sources, a total of 53 sites on 24
lakes within a 100 km. radius of Balsam Lake were surveyed.
Clam species were identified, their population densities
estimated and the site accessibility and environmental
characteristics assessed. From this information, the
possibility of utilizing alternate sources and species of
clams for biomonitoring purposes was examined.

METHODS

The objective of our survey was to examine a variety of environmentally different lakes within a 100 km. distance from Balsam Lake and with good accessibility.

Lake selections were based on a combination of factors, including pH, geographic area, hydrotopography, watershed, and proximity to potential pollution sources. This information was obtained from MOE Water Quality Data Vol. XVI, Canadian Topographic Series maps, provincial maps, nautical maps, geological maps, local sources and personal communications. From this information, a number of lakes considered most representative, were chosen as potential survey areas.

Survey sites within the lakes were then chosen by examining inflow areas on the lake, hydrotopography (when available), proximity to contaminant sources (towns, marinas, dumps etc.) and boat accessibility.

Inflow areas and shallow bays were avoided as they were assumed to contain a high level of unstable sediment deposits and usually heavy plant growth, conditions considered unfavourable for clam populations according to our past field experience. Areas just beyond littoral zones and in line with flow direction, were considered to have good potential for clams as the nutrient levels would still be high but substrates would be more stable.

Shorelines with steep drop-offs to depths where clam beds are not found (approximately 30 feet and deeper) were not considered. If all conditions were ideal but the area was remote and not easily accessible, it was not considered for survey because of time and other practical constraints.

After these initial selections were completed, an itinerary was developed that incorporated the greatest number of lakes and survey points in the most efficient expense and time frame possible. Survey lakes and points were priorized according to accessibility and how representative of the area they were, so that the schedule could be adjusted as necessary in the field.

Equipment for the survey included a 4.5 metre aluminum boat with 45 hp. motor, S.C.U.B.A. equipment, AGA masks and radio communications, tow sled and line, bags and bottles for sample collection and appropriate reference and recording materials.

Once sites were located and accessed, they were surveyed
utilizing a combination of equipment and techniques,
dependent upon the depth, current, visibility, and
presence/absence of beds. Survey areas were a minimum of
5,000 square metres and a maximum of 12,500 square metres
when substantial beds were located. The following density
scale was used:

> #1 - 5 clams/sq.m.
> #2 - 10 clams/sq.m.
> #3 - 50 clams/sq.m.
> #4 - 100 clams/sq.m.
> #5 - $\geqslant$ 150 clams/sq.m.

Clam density was estimated using metre squares, samples were
taken and alternative species searched for. Site information
including substrate type, current, depth etc. was recorded
and at least three clams were sampled per site for future
species confirmation, ageing and overall examination.

CLAM R & D FIELD SURVERY - ITINERARY

<u>DAY 1 - July 1, 1987</u>

Cameron Lake   1-1 Rathbun Island
                            1-2 Northeast Point
                            1-3 Gregory Point

Sturgeon Lake   2-1 Stinson Bay
                            2-2 Hawkers Bay
                            2-3 Hotel/Boys Camp
                            2-4 Muskrat Island
                            2-5 McConnel Island
                            2-6 Ancona Point

Lower Buckhorn Lake   3-1 Bay north of town
                            3-2 Deer Creek Bay
                            3-3 Three Islands west shore
                            3-4 Offshore Marinatha Marina

<u>DAY 2 - July 2, 1987</u>

Shadow Lake   4-1 Island east of trailer park
                            4-2 North point at river mouth

Head Lake   5-1 West side at gravel launch
                            5-2 Southern end beach

Moore Lake   6-1 Below Moore Falls

Gull Lake   7-1 Easter Island
                            7-2 Harriet Island
                            7-3 North point near launch

<u>DAY 3 - July 3, 1987</u>

Horseshoe Lake   8-1 North end at Cupboard Gen. Store
                            8-2 Coburn Island

* Mountain Lake not accessible by boat

Soyers Lake   9-1 Browns Island - southern tip

Kashagawigamog Lake   10-1 South of Soyers inflow
                            10-2 Western shore near lodge

Canning Lake   11-1 Ingoldsby Bay
                            11-2 East of Battle Island
                            11-3 Central area
                            11-4 Southern tip (Bat Lake Rd.)

DAY 4 - July 4, 1987

Haliburton Lake	12-1	North channel near Marina
	12-2	Btwn. mainland and 1st island
	12-3	Southern tip of third island
	12-4	Northern tip of third island
	12-5	West side of third island
Eagle Lake	13-1	Below Redstone River Dam
Green Lake	14-1	North shore Restaurant area
	14-2	South shore
Gull River	15-1	Between Maple and Green Lake
Maple Lake	16-1	North side lodge area
Beech River	17-1	At mouth to Boshkung Lake
Boshkung Lake	18-1	Bay at Beech River inflow
	18-2	At mouth to Little Boshkung (above, at junction and below)
Twelve Mile	19-1	At junction with Little Boshkung

DAY 5 - July 5, 1987

Halls Lake	20-1	Kenesis River at mouth to Halls
	20-2	Bay on east side of point
	20-3	Above Buttermilk Falls
Kushog Lake	21-1	At Little Kushog junction (above, at junction and below)
St. Nora Lake	22-1	Bay at Leslie Frost Res. Station
	22-2	Northwest Point
Lake of Bays	23-1	Dorset
Harp Lake	24-1	At boat launch
	24-2	Opposite side of lake

TOTALS: **24 Lakes**
53 Survey Points
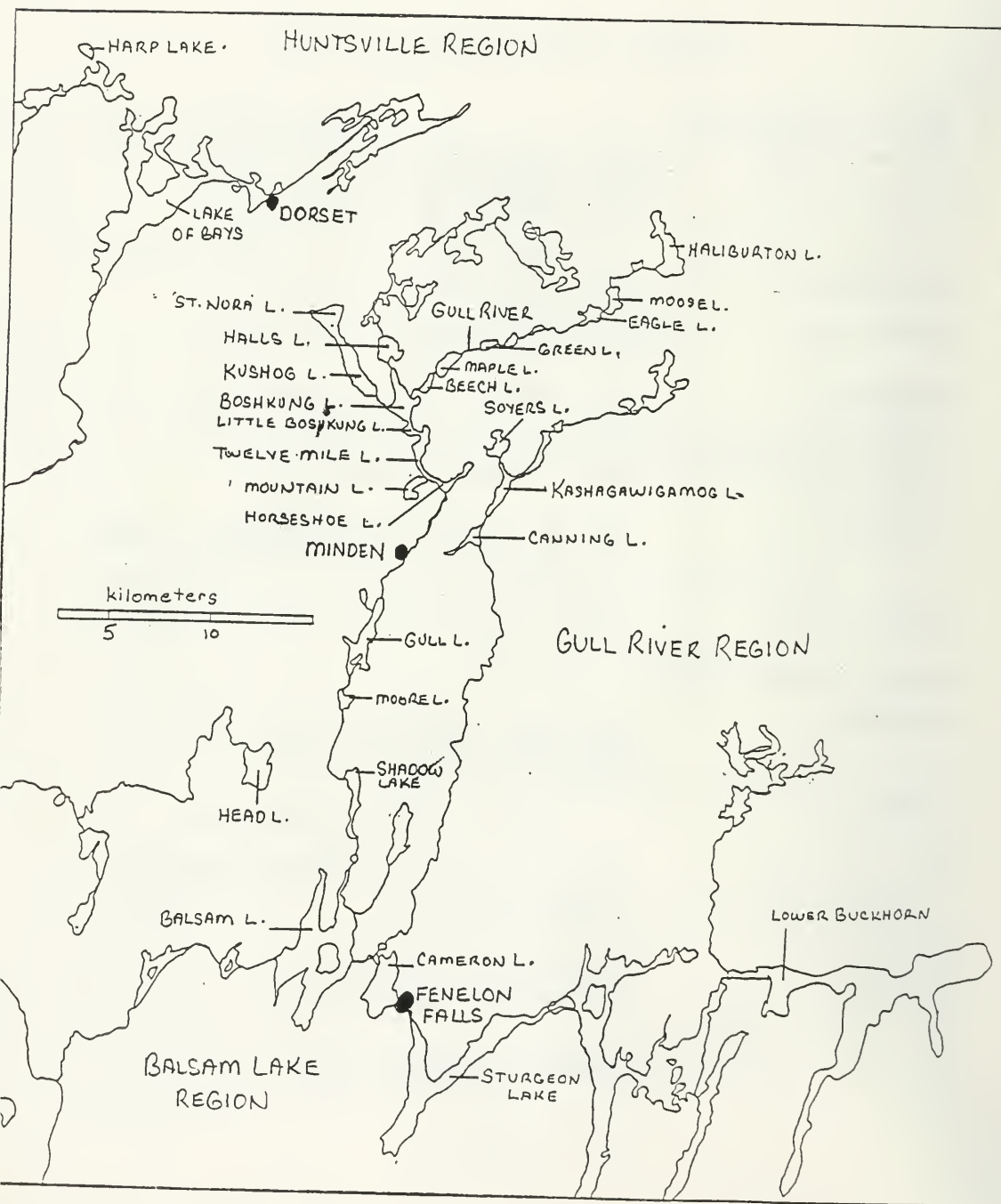
Figure 1. Map of Survey areas.

Table 1. <u>LAKES SURVEYED FOR UNIONID CLAMS - JULY, 1987</u>

LAKE	TOWNSHIP	LATITUDE	LONGITUDE
Cameron	Fenelon	44 32'	78 45'
Sturgeon	Verulam	44 27'	78 40'
Buckhorn	Burleigh/Harvey	44 33'	78 10'
Shadow	Laxton	44 42'	78 47'
Head	Laxton	44 43'	78 55'
Moore	Lutterworth	44 47'	78 47'
Gull	Lutterworth	44 49'	78 45'
Horseshoe	Minden	44 59'	78 41'
Soyers	Minden	45 02'	78 30'
Kashagawigamog	Minden/Dupart	45 00'	78 33'
Canning	Minden/Snowdon	44 52'	78 38'
Haliburton	Harburn	45 11'	78 23'
Eagle	Guilford	45 07'	78 27'
Green	Guilford	45 06'	78 36'
Gull (River)	Stanhope	45·06'	78 38'
Maple	Stanhope	45 06'	78 40'
Beech (River)	Stanhope	45 05'	78 43'
Boshkung	Stanhope	45 04'	78 44'
Twelve Mile	Minden	45 02'	78 43'
Halls	Stanhope	45 07'	78 45'
Kushog	Stanhope	45 07'	78 48'
St. Nora	Herborne/Stanhpoe	45 11'	78 51'
Lake of Bays	Ridout	45 14'	78 54'
Harp	Chaffey	45 22'	79 07'

OBSERVATIONS AND RESULTS

The clam species <u>Elliptio</u> <u>complanata</u> was found, to varying
degrees, in virtually every water body surveyed. Other
species such as <u>Lampsilis</u> and <u>Anodonta</u> were observed, but
nowhere in densities great enough to permit routine
harvesting for biomonitoring or research purposes. The
incidence of these two latter species appeared to increase in
a northerly progression.

Three survey sites harboured <u>Elliptio</u> clam populations with
densities of greater than 100 clams per square metre, and
therefore were considered as excellent alternative sampling
sources. Approximately ten sites had densities of between 50
and 100 clams per square metre as well and could also be
considered adequate supply sources. All of these sites .
contained specimens of the appropriate size class now used
for biomonitoring purposes and the retrieval time of routine
sample sizes (200 to 300 clams) would be minimal for a diver.

Substrate was not as determining a factor as originally
thought in clam distribution as long as it was stable enough
to allow anchoring of the clams. For example, silty areas
that were overlying sand or silty areas with abundant, short
macrophytic growth, were both suitable conditions for clams,
as were rocky areas with silty, sandy patches in between.

Macrophytic growth occasionally played a determinant role in
the presence of clam beds in two ways. Tall, dense stands of
macrophytes tended to restrict the clam distribution while
shorter, sparse stands provided substrate stability,
therefore anchoring support for the clams.

Observations indicated that current could be a key factor
determining clam distribution. A fairly strong current
allowed continuous transport of suspended nutrient material
for food, but prevented anything but minimal settling of
debris, silt and mud that would tend to bury or clog the
clams. The current also supplied an abundance of oxygen.
These two factors could be responsible for a lack of
competition between clams, therefore encouraging very dense
growth. However, a current that did not carry suspended
material, such as in the Kenesis River, did not support any
clam populations however high the oxygen content. Current
has probably also served to increase their geographical
range. Clams with unusually polished outer surfaces were
found in the lower reach of the Gull River entering Boshkung
Lake. These could indicate that some clams may have been
transported by being tumbled down river.

Areas directly around infow sources, were consistently found
to have conditions unsuitable for clam populations. These
littoral zones were almost always characterized by heavy
sedimentation, abundant macrophytic growth and high organic
sediments. Just beyond these zones, the conditions were
consistently favourable, as the nutrient level was high, but
the sediments were less organic and also more stable.

Rivers seemed to provide the most ideal environment for
supporting dense clam beds, as long as the water quality was
appropriate. From our subjective observations it would
appear that both high nutrient and oxygen levels are required
to support the very dense clam beds that were encountered
during our survey.

pH values ranged between 6.2 and 8.0 in the lakes we surveyed
and did not appear to be a determining factor in clam
distribution in these cases.

A low level of calcium carbonate in the water could be
expected to limit clam distribution and this parameter would
be reflected as a low value on an alkalinity scale. Of all
lakes surveyed, Halls Lake had the lowest value of 2 and an
almost negligible clam population.

Clams were observed in dense populations at depths ranging
from 1.5 metres to 5 metres, therefore precluding depth as a
determinant factor in these cases.

Table 2. Field data

LAKE	SITE	CLAM DENSITY	(*) C/P	LOCATION OF BEDS: WATER DEPTH (metres)	pH(**)	CLARITY (metres)	SUBSTRATE	CURRENT
Cameron	1-1	4	C	0.5-2.0	7.8(5)	2.0	sand/pebbles/rock	none
Cameron	1-2	3	C	0.5-2.0	7.8(5)	2.0	sand	none
Cameron	1-3	2-3	P	2.5	7.8(5)	2.0	rocks	none
Sturgeon	2-1	0	-	-	7.9(5)	1.0	coarse sand/macrophytes	none
Sturgeon	2-2	0	-	-	7.9(5)	1.0	coarse sand/macrophytes	none
Sturgeon	2-3	1/4	P	1.5-2.0	7.9(5)	2.0	sand/rock	none
Sturgeon	2-4	0	-	-	7.9(5)	2.0	coarse sand/rock/macroph.	none
Sturgeon	2-5	0	-	-	7.9(5)	2.0	coarse sand/rock/macroph.	none
Sturgeon	2-6	0	-	-	7.9(5)	1.5-2.0	boulders/cobble	none
L. Buckhorn	3-1	0	-	-	8.0(5)	1.0	sand/pebbles	none
L. Buckhorn	3-2	1/2	P	2.0	8.0(5)	1.5	silt	slow
L. Buckhorn	3-3	1	P	2.5	8.0(5)	2.5	sand/boulders/cobble	none
L. Buckhorn	3-4	1/2	P	2.5-3.0	8.0(5)	1.5	sand/large boulders	none
Shadow	4-1	3	C	2.0	7.2(4)	3.0	sand/rock/dense algae	none
Shadow	4-2	3	C	3.0 max	7.2(4)	3.0-3.5	silt/sand	slow
Head	5-1	0	-	-	7.7(4)	0.5-1.0	silt/detritus/macrophytes	none
Head	5-2	1-1½	P	1.0	7.7(4)	0.5-1.0	fine silt/detritus	none
Moore	6-1	1/2	P	2.5-3.0	7.1(4)	3.5	organic mud/silt/sand	slow
Gull	7-1	2-3	C	3.0-6.0	7.1(4)	4.5	silt/sand/macrophytes	slow
Gull	7-2	3	C	3.0-6.0	7.1(4)	6.0	sand/silt	slow
Gull	7-3	1-2	P	4.5-6.0	7.1(4)	4.5	sand/silt	none
Horseshoe	8-1	1/2	P	3.0 max	7.1(3)	3.0	silt	none
Horseshoe	8-2	1-2	P	2.0-4.5	7.1(3)	4.5	silt/sand/algae	moderate
Soyers	9-1	1/2	P	2.0-6.0	7.1(3)	2.0-2.5	rock/silt/sand	none
Kashagawig.	10-1	1/2	P	1.5-4.5	7.1(4)	1.5	silt/sand/macrophytes	none
Kashagawig.	10-2	1/2	P	1.5-4.5	7.1(4)	1.5 max	silt/weeds	none
Canning	11-1	1/4	P	3.0 max	7.1(4)	1.5	silt/detritus	none
Canning	11-2	2	P	3.0 max	7.1(4)	1.5	silt/sand	none
Canning	11-3	2	P	3.0-4.5	7.1(4)	1.5	silt/sand/drop-off	slow
Canning	11-4	2	C	3.0	7.1(4)	0.5-1.5	silt/sand/bedrock	none
Haliburton	12-1	1½-2½	P	2.5-3.0	7.3(3)	2.5	sand bar	none
Haliburton	12-2	1-1½	P	2.5-4.5	7.3(3)	2.5	sand bar	slow
Haliburton	12-3	1/2	P	2.5-4.5	7.3(3)	3.5	silt/sand/drop-off	none
Haliburton	12-4	1/2	P	2.5-4.5	7.3(3)	3.5	silt/sand/drop-off	none
Haliburton	12-5	2-2½	C	2.5-3.0	7.3(3)	3.0-3.5	pebbles/silt/sand	none

Table 2 - continued

LAKE	SITE	CLAM DENSITY	(*) C/P	LOCATION OF BEDS: WATER DEPTH (metres)	pH(**)	CLARITY (metres)	SUBSTRATE	CURRENT
Eagle	13-1	1	C	1.0-3.5	7.1(4)	2.0	humus/silt/sand	slow
Green	14-1	1-1½	C	1.0-2.0	7.5(4)	2.0	silt/macrophytes	none
Green	14-2	1-2	C	2.0-3.0	7.5(4)	2.0	silt/stable mud	none
Gull River	15-1	5	C	1.5-4.5	-(4)	2.5	silt/weeds	moderate
Maple	16-1	1/2	P	1.5-3.0	7.1(4)	1.0	silt	none
Beech River	17-1	4	C	1.5-3.0	-(4)	2.5	rocks/sand	strong
Boshkung	18-1	2-3	P	0.5-4.5	6.9(3)	2.5-3.0	sand/rock	none-modera
Boshkung	18-2a)	2-3	C	2.5-3.0	6.9(3)	3.0	sand/silt/weeds	moderate
Boshkung	18-2b)	0	-	-	6.9(3)	3.0	sand/silt/weeds	moderate
Boshkung	18-2c)	1½	P	2.5-3.0	6.9(3)	3.0	sand/silt/weeds	slow
Twelve Mile	19-1	0	-	-	6.8(3)	3.0-3.5	silt/algae	slow
Halls	20-1	1/2	P	2.0-3.5	6.6(2)	10.0 min	silt/sand/wood detritus	strong
Halls	20-2	1/4	P	3.0-8.0	6.6(2)	10.0 min	sand/silt/drop-off	none
Halls	20-3	1/2	P	1.0-3.0	6.6(2)	10.0 min	sand/rock	moderate
Kushog	21-1a)	1	P	3.0-6.0	6.8(3)	4.5	silt/sand/rocks	slow
Kushog	21-1b)	1/2	P	8.0-9.0	6.8(3)	4.5	silt/sand/rocks	none
St.Nora	22-1	1	C	1.5-3.0	6.2(3)	1.5	sand/pebbles/silt	none
St.Nora	22-2	2½	C	3.0-9.0	6.2(3)	1.5-2.0	silt/clay	none
Lake of Bays	23-1	3	C	2.0	6.7(3)	2.5	sand/rocks	slow
Harp	24-1	0	-	-	6.3(3)	1.0	detritus/silt/drop-off	none
Harp	24-2	1-2	C	1.5-4.5	6.3(3)	0.5-1.0	sand/silt/detritus	none

* C = Continuous distribution
  P = Patchy distribution

** Alkalinity value

CONCLUSIONS

From this survey we conclude the following:

1. <u>E. complanata</u> would be the species of choice for use
   in biomonitoring surveys due to its wide distribution
   and occurrence in sufficient densities to permit
   practical harvesting.

2. The distribution pattern of <u>E. complanata</u> within a
   lake is governed by numerous factors that cannot be
   determined by subjective observation alone. However
   it is strongly suspected that both high levels of
   nutrient and oxygen tension in conjunction with substrate
   stability are required to support dense clam populations.

3. The pH and alkalinity range to which <u>E. complanata</u>
   can be subjected makes it suitable for monitoring a
   wide range of water bodies down to a pH of 6.2 and
   alkalinity value of 3.

MAP KEY

3-1   Survey Site

$2\frac{1}{2}$ Clam Density Rating

        General Direction of Reconnaissance
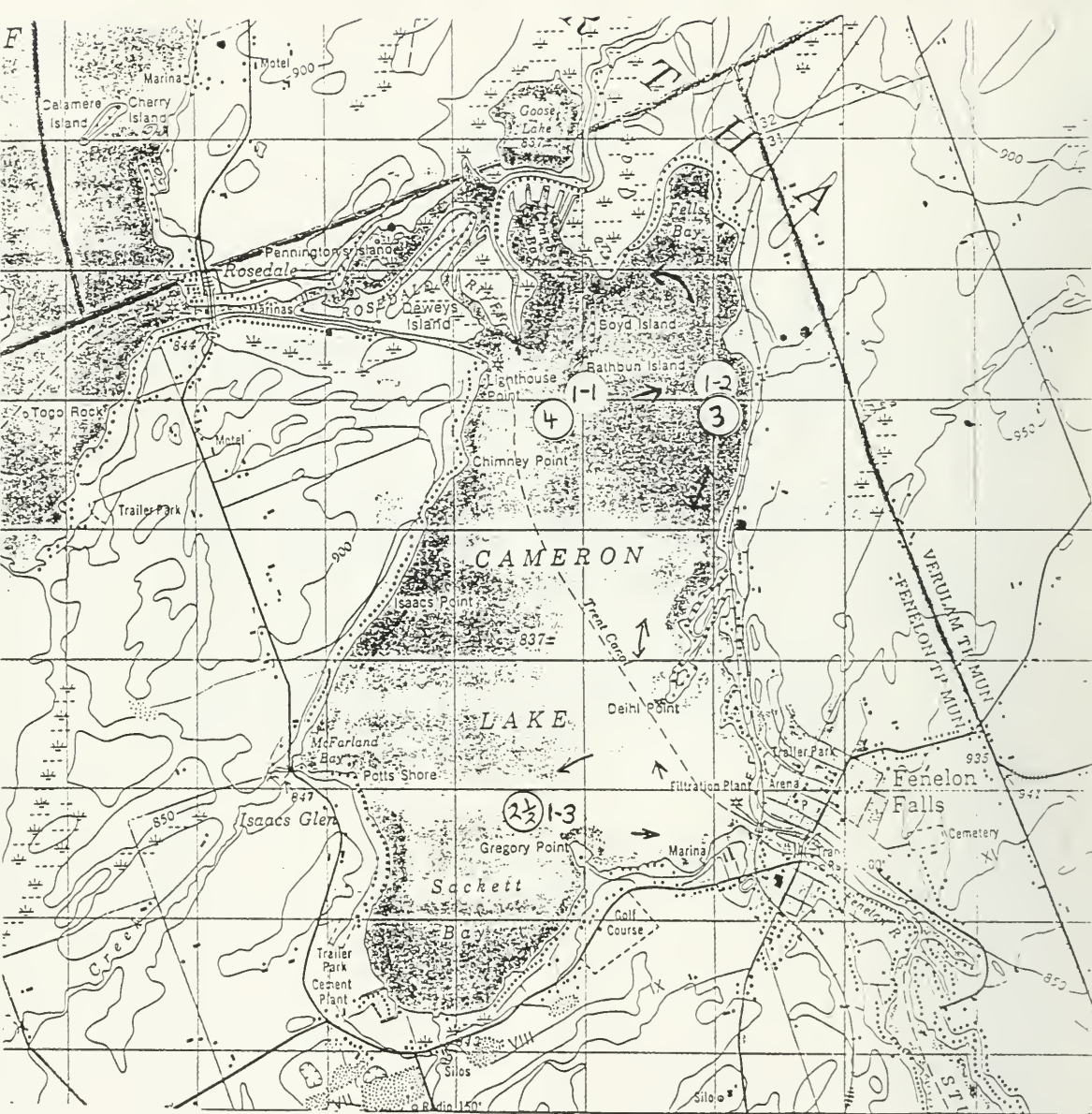
CAMERON LAKE - July 1, 1987

Boat access: At southeast marina location.

1-1: Clams thickest at east side of island, very scarred in appearance. large amounts of freshwater sponge and crayfish. _Anodonta_ observed here.

1-2: Clams not as scarred, continuous distribution, _Lampsilis_ observed here.

1-3: Drop-off, no clams observed at depths greater than 3.5 m.
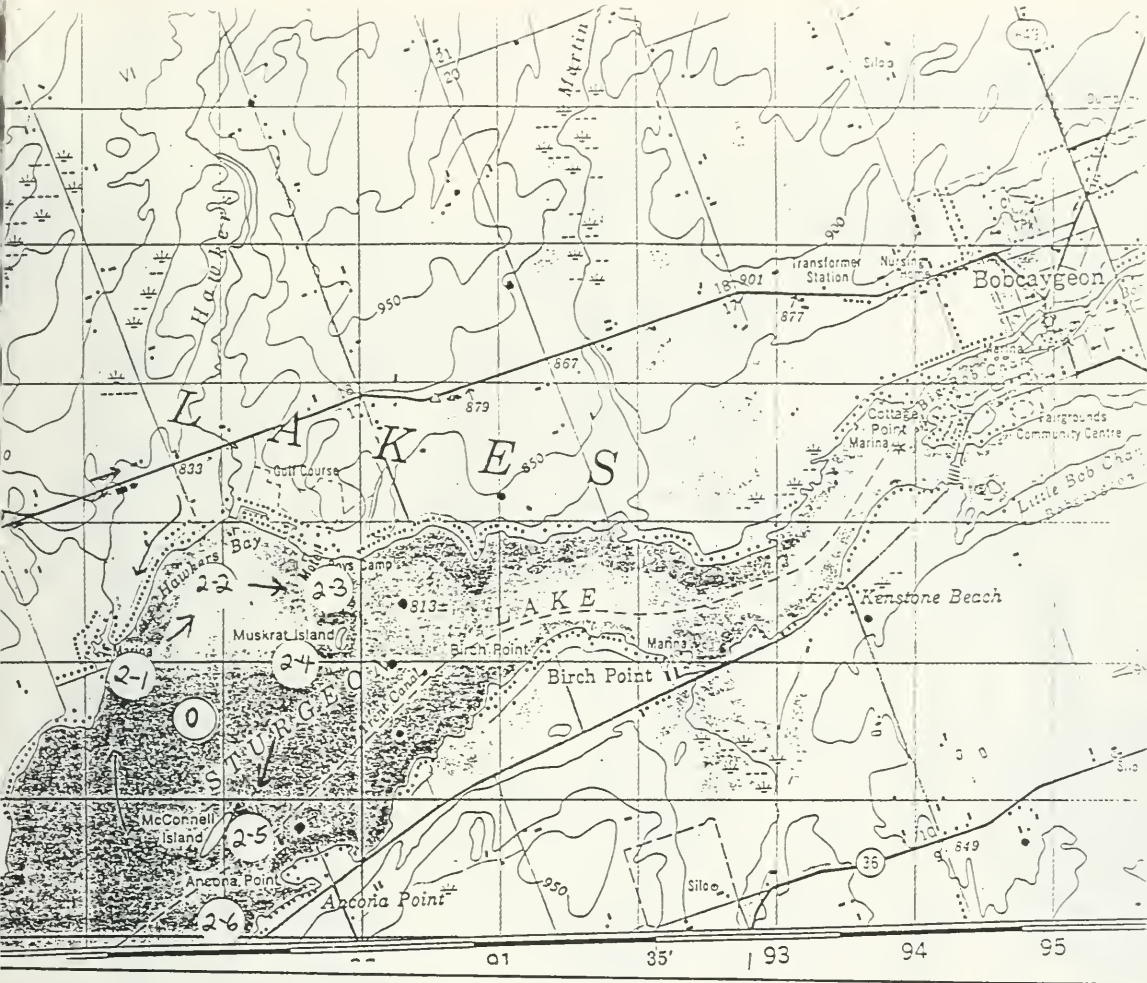
STURGEON LAKE - July 1, 1987

Boat access: Off ramp at end of Hawkers Bay Rd. (Stinson Bay)

2-1: Abundant Chara and suspended material

2-4, 2-5: Dense Myriophyllum (millfoil).

LOWER BUCKHORN LAKE - July 1, 1987

Boat access: Central Buckhorn marina or Marinatha marina (south shore).
              None on the northern shore.

3-2: Many empty shells observed - flesh eaten by muskrats.
      Anodonta shells observed.
      Broad leafed plants, including Potamogeton.
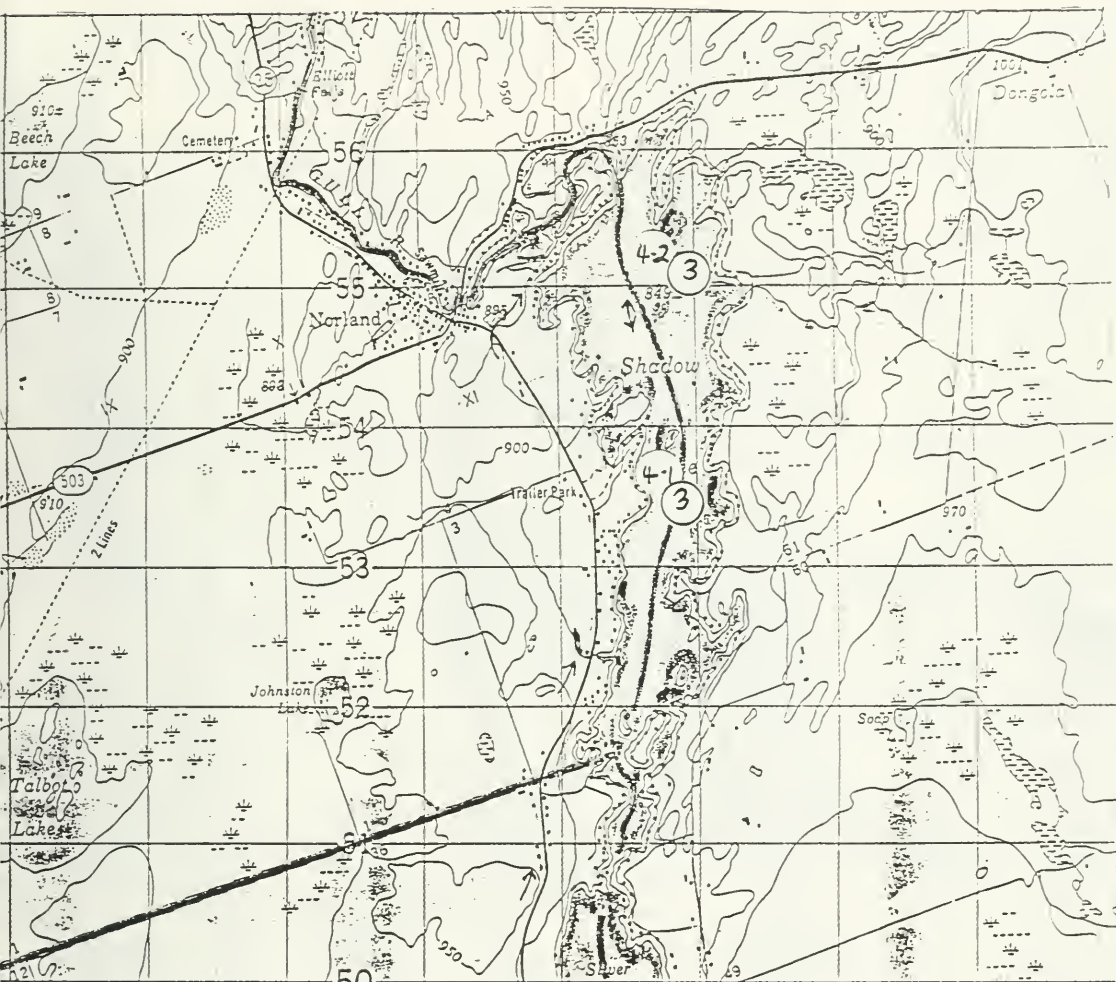
SHADOW LAKE - July 2, 1987

Boat access: Government dock - northwest side road

4-1: dense algal mat covering rocks
      abundant Najas and Isoetes (quillwort)
      Anodonta observed here

4-2: fewer macrophytes, more silt and sediment
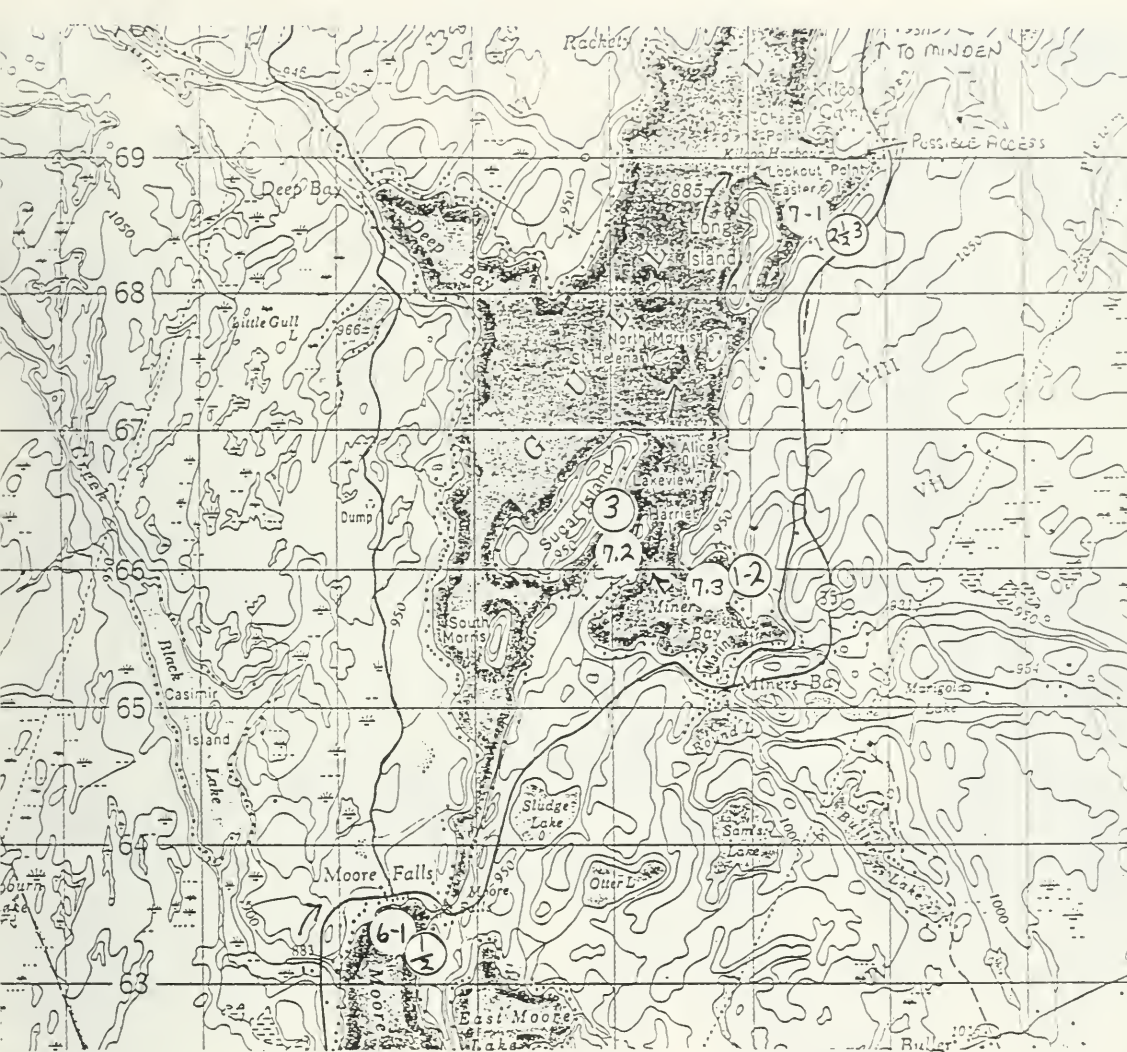   denser clam beds on east side of penninsula
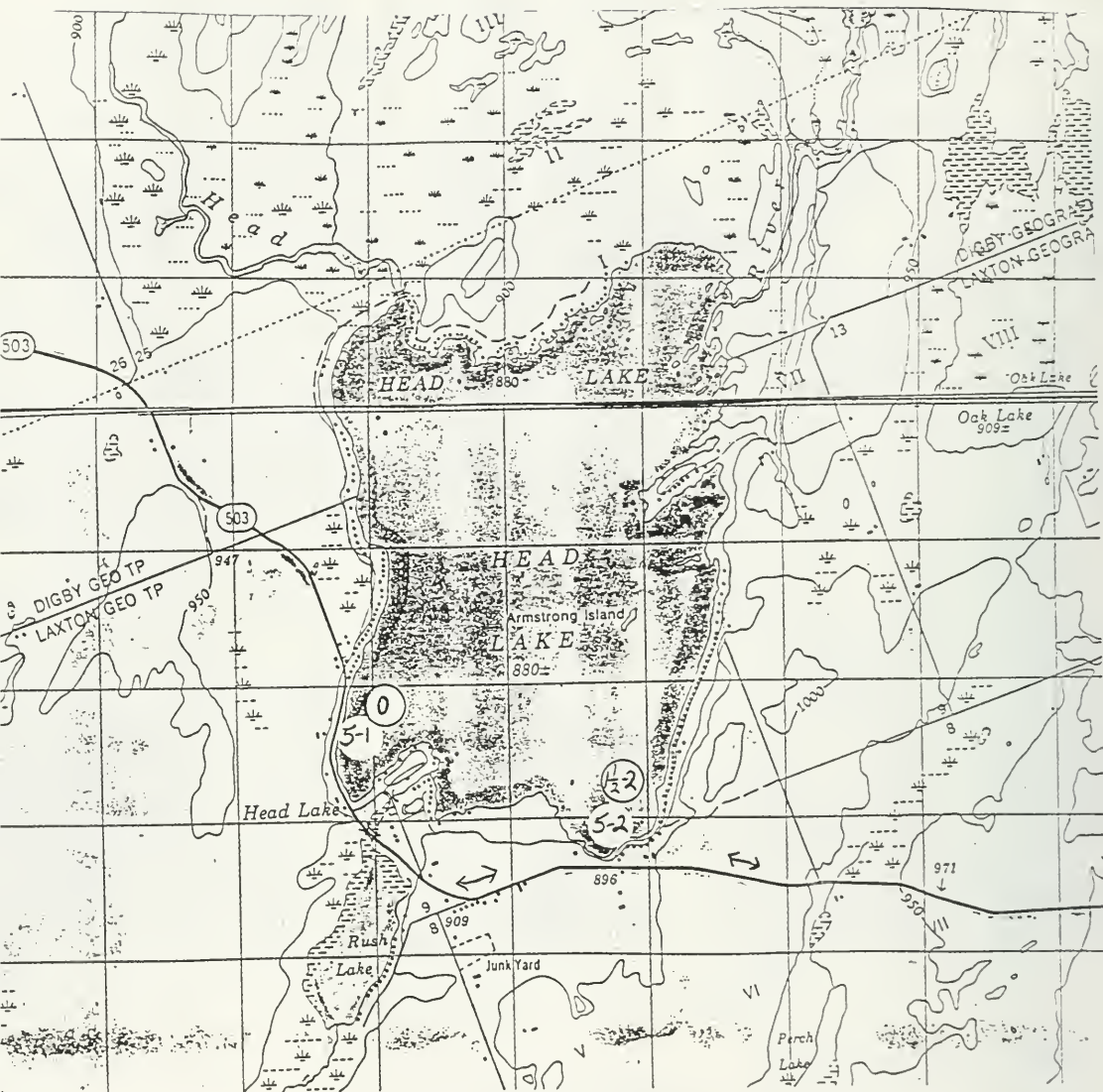
HEAD LAKE - July 2, 1987

Boat access: South shore sandy beach.   No practical access anywhere else
               (western shore has steep, loose gravel launch).

5-2:   Abundance of macrophytes
   Very muddy substrate at greater than one metre
       Clam density increases in eastern direction and decreases in western direction

MOORE LAKE AND GULL LAKE - July 2, 1987

Boat access: (Gull L.) Marina on southerly bay of Miners Bay and possible sites at
              Braeside Cottages and Kilcoo Camp on Hwy. 35.
              (Moore L.) Marina on north shore beside falls.

6-1: · steep drop-off close to shore.
      sparse macrophytes.

7-1: Clam density higher on southeast shore area of island.
     abundant vascular plants including _Isoetes_ and _Vallisneria_. ·

7-2: Continuous snail beds (_Viviparus_) along with clams at 4-5 m.
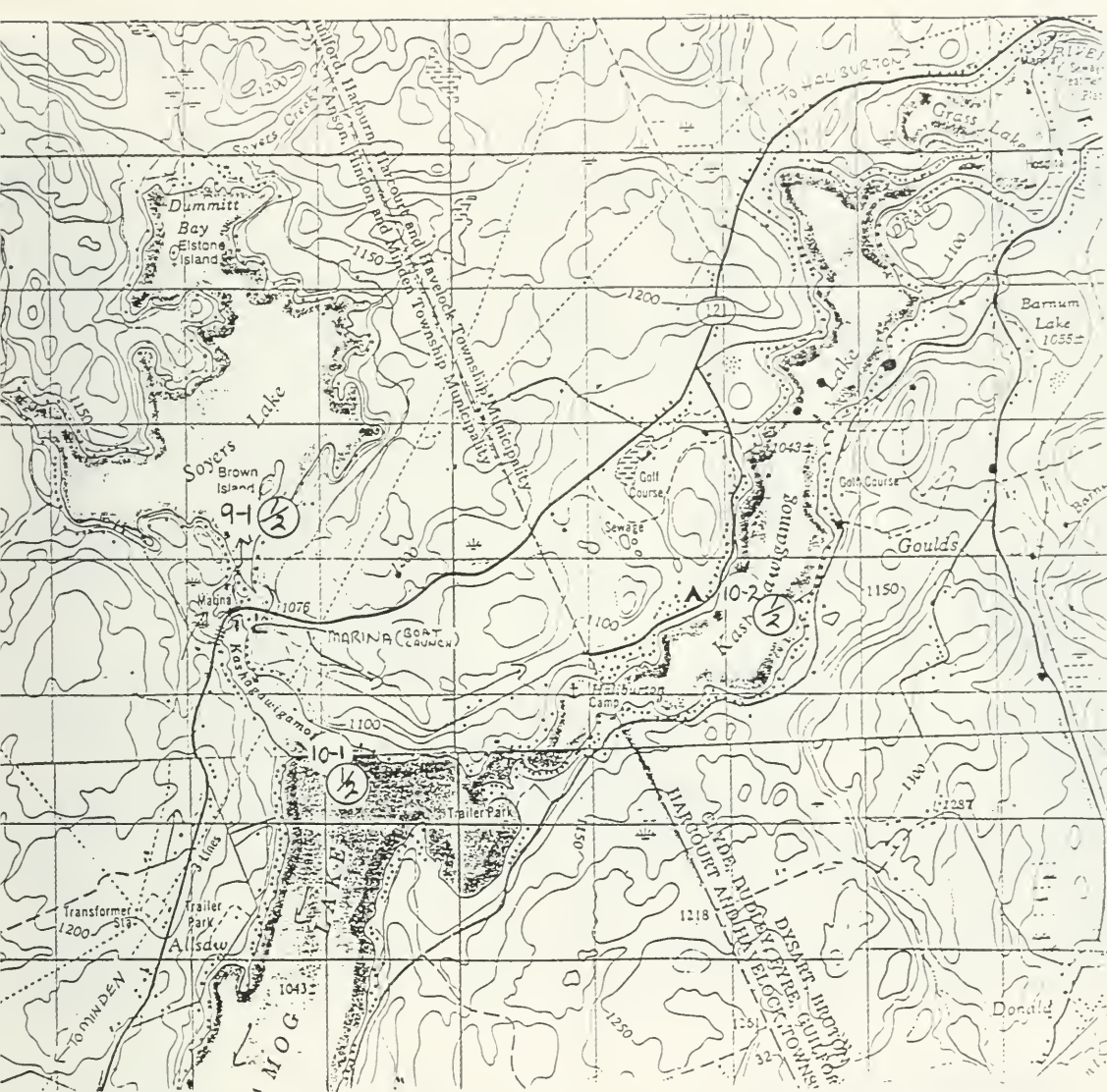   _Eliptio_ specimens varied greatly in size.
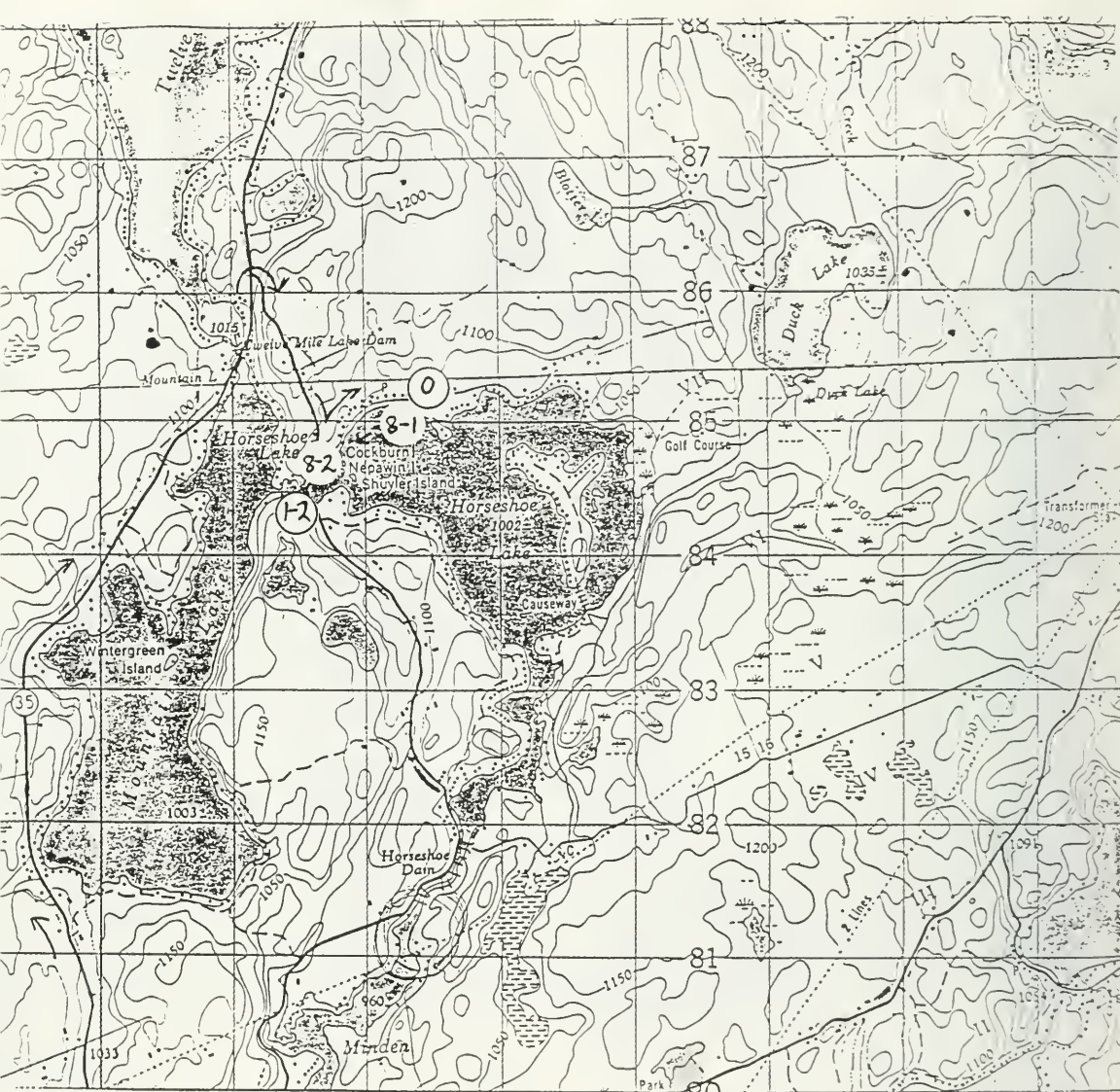
HORSESHOE LAKE - July 3, 1987

Boat access: Across from Caupboard General Store, north shore.

8-1: Lots of empty shells (muskrat feeding).

8-2: Denser beds observed in channel between island and shore where sand available.
      Clouds of algae observed.

SOYERS LAKE AND KASHAGAWIGAMOG LAKE - July 3, 1987

Boat access: Marina south of HWY 121.

9-1: <u>Eliptio</u> small size.
      Little macrophytic growth.

10-1: Abundant macrophytic growth.

10-2: Large variety of vascular plants at shallow depths.

CANNING LAKE - July 3, 1987

Boat Access (11-1, 11-2, 11-3) Marina below HWY 121 at intersection with Soyers Lake.
   Access (11-4) Bat Lake Rd., east from Minden (no boat access).

11-1: very silty, large amount of wood and leaf detritus.
        Patchy _Isoetes_.

11-2, 11-3: Abundant macrophytic growth in shallow water.

11-4: _Anodonta_ observed (#1 rating).
        Poor visibility due to intense afternoon storm.
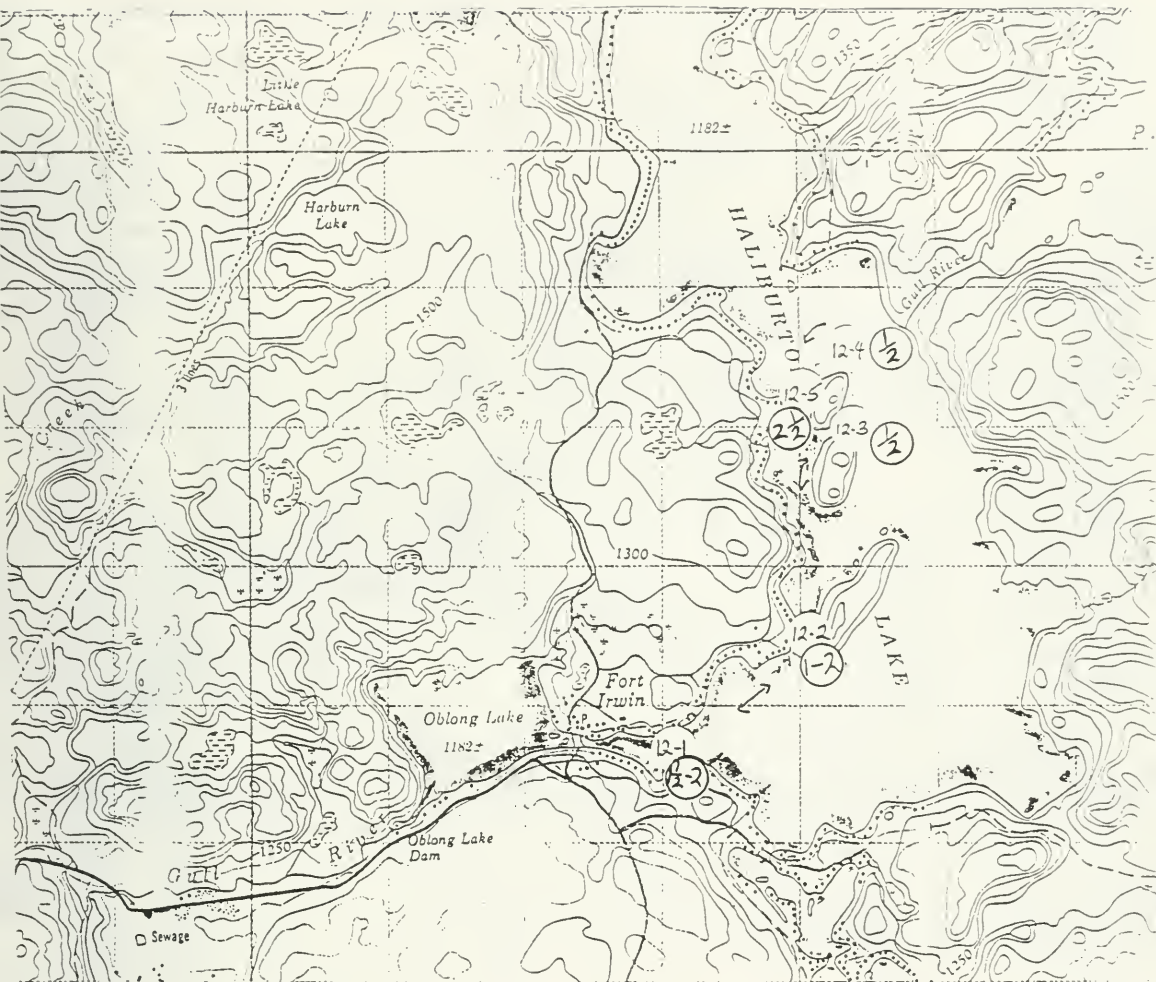
HALIBURTON LAKE - July 4, 1987

Boat access: Fort Irwin Marina.

12-1:   Lots of empty clam shells.
        Substantial rock barrier at 1 1/2 to 2 m.
        No vascular plants.

12-2:   Sand bar between island and mainland.

12-3, 12-4: Steep drop-offs.

12-5:   Clams large in size.
        Abundant freshwater sponge.
        Sparse _Isoetes_.
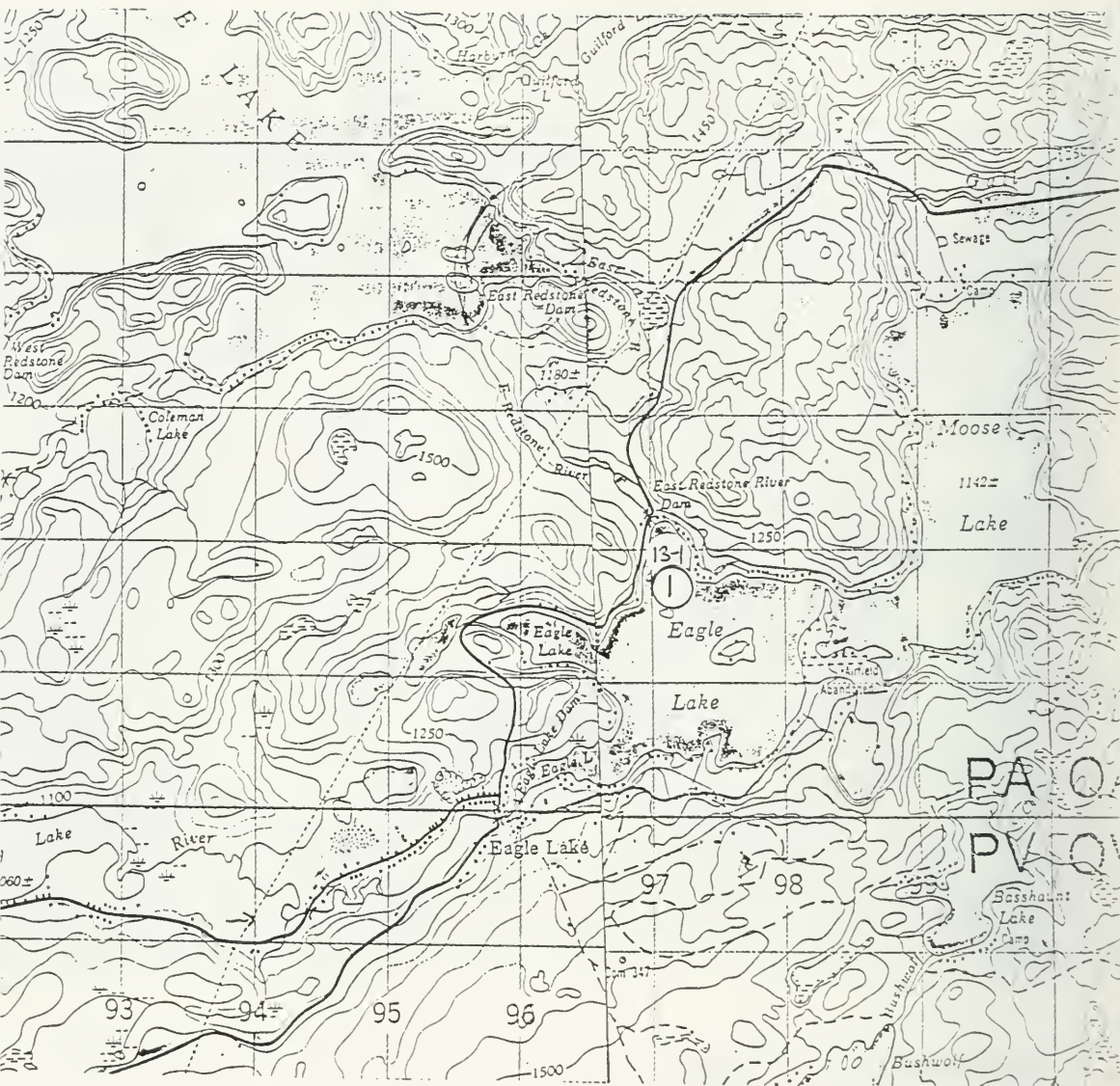
EAGLE LAKE - July 4, 1987

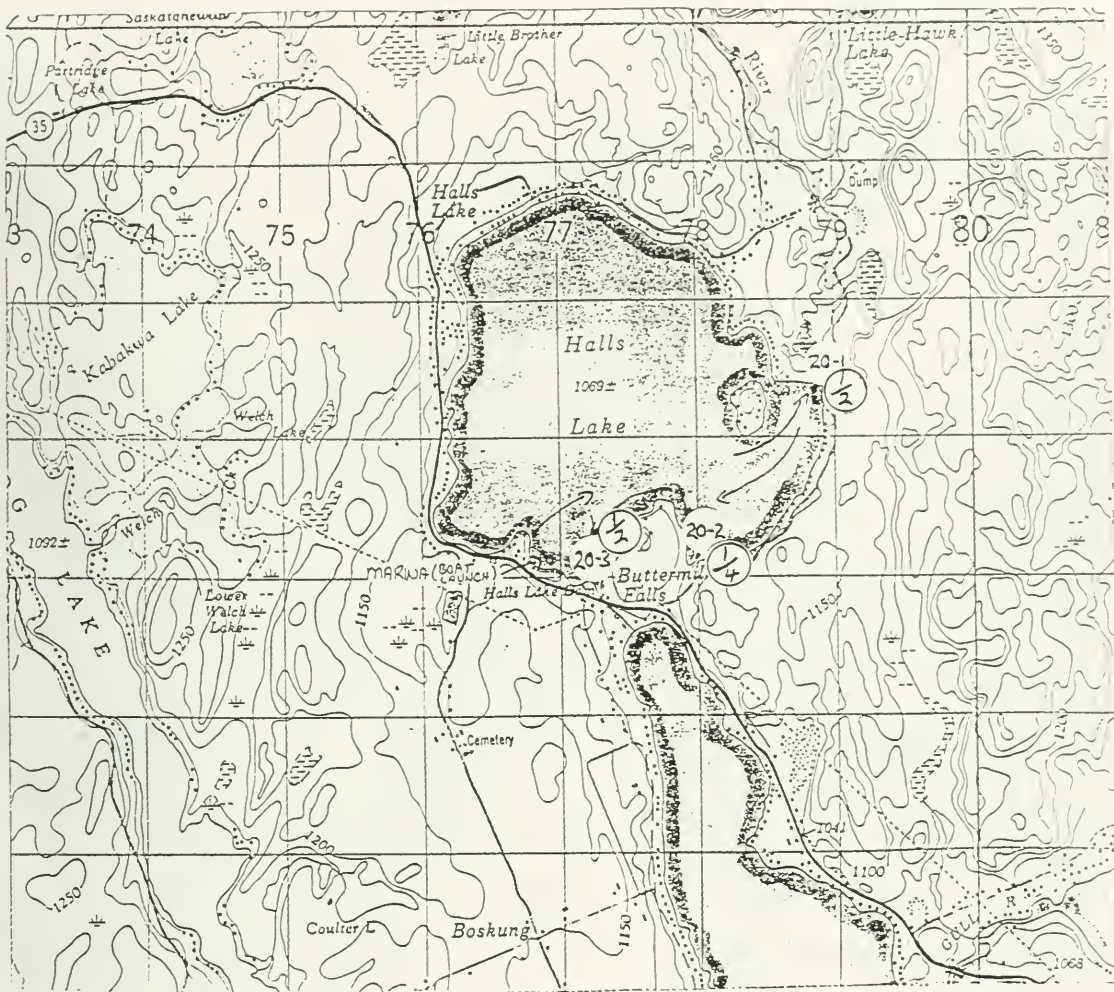Access: (diver only) Off road below East Redstone River Dam.

13-1: Small _Isoetes_, no observable algae.
       Abundant bass
       Humus and leaf and wood detritus on bottom.
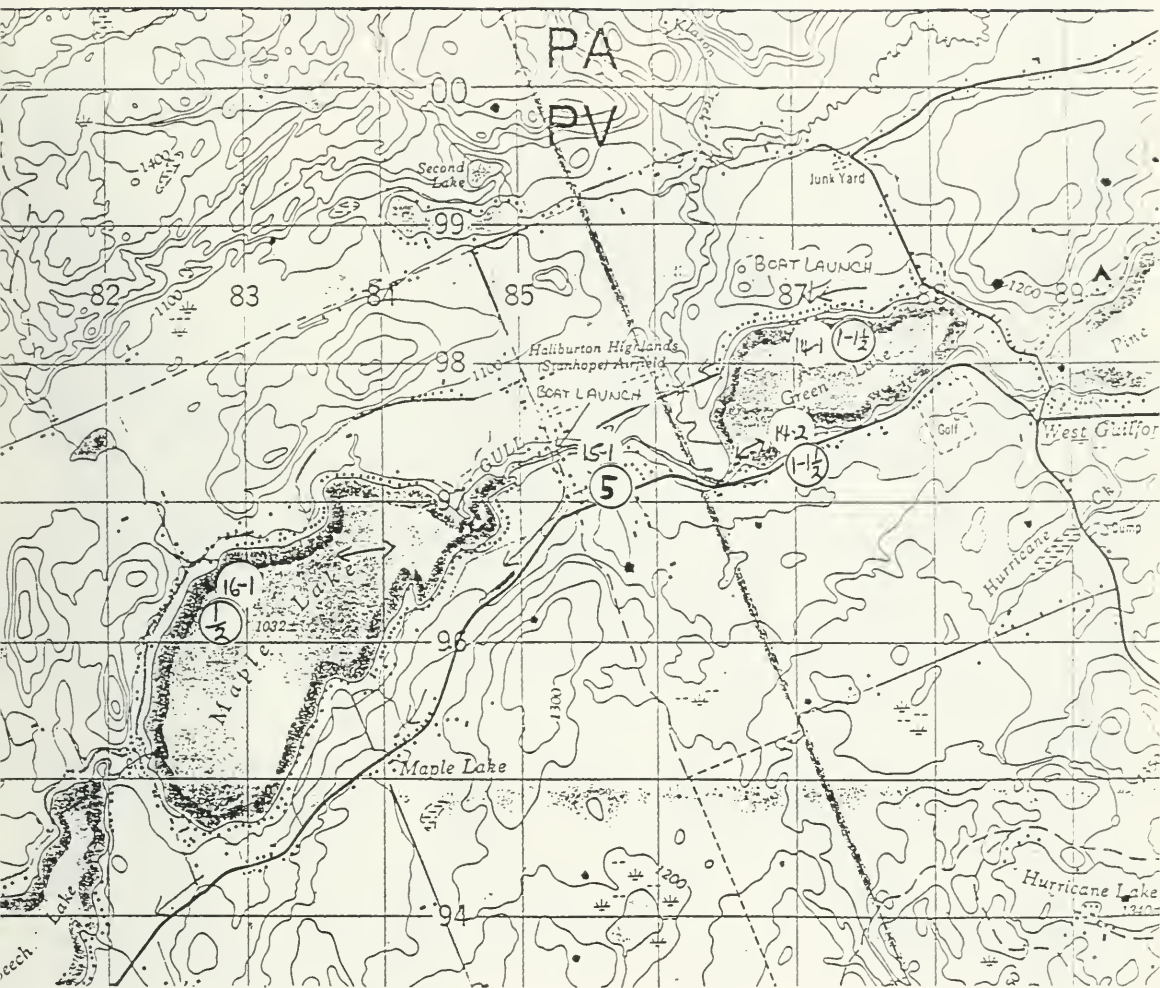
GREEN LAKE, GULL RIVER , MAPLE LAKE - July 4, 1987

Boat access: Across from north shore restaurant on Green Lake
              Boat launch beside bridge over Gull River (HWY 118 to Maple Lake
              United Church, turn left to bridge).

14-1: Shallow water throughout lake.

14-2: Abundant _Valisneria_, _Isoetes_ and rhizomatous material.
       Eliptio various sizes.

15-1: Bottom silty and weedy, abundant _Isoetes_.
       Maximum depth 3-4 m., moderate current.

16-1: Clams scarce but all near vascular plants.

BEECH RIVER, BOSHKUNG, LITTLE BOSHKUNG AND TWELVE MILE LAKE - July 4, 1987

Boat access: Marina above Twleve Mile Lake Dam.

17-1: Clams positioned sideways, shells polished in appearance, but many broken.
       Current fairly strong, a lot of wood detritus.

18-1: Density variable with current, greater in stronger current, less in
       shallow, still areas.

18-2: Density decreased: #3 above bridge, #2 under, and #1/2 below.
       Found specimen with shell dissolved except for periostracum.

19-1: Dense mat of plants with algal netting over the top.

HALLS LAKE - July 4, 1987

Boat Access: Southern Marina, entrance off HWY 35 above Buttermilk Falls.

20-1: Clarity excellent (greater than 10 m.)
       Wood detritus and sparse macrophytic growth on bottom.
       Schools of suckers.
       Clams covered with silt, difficult to see.

20-2: Sparse macrophytes.
       Steep drop-off.
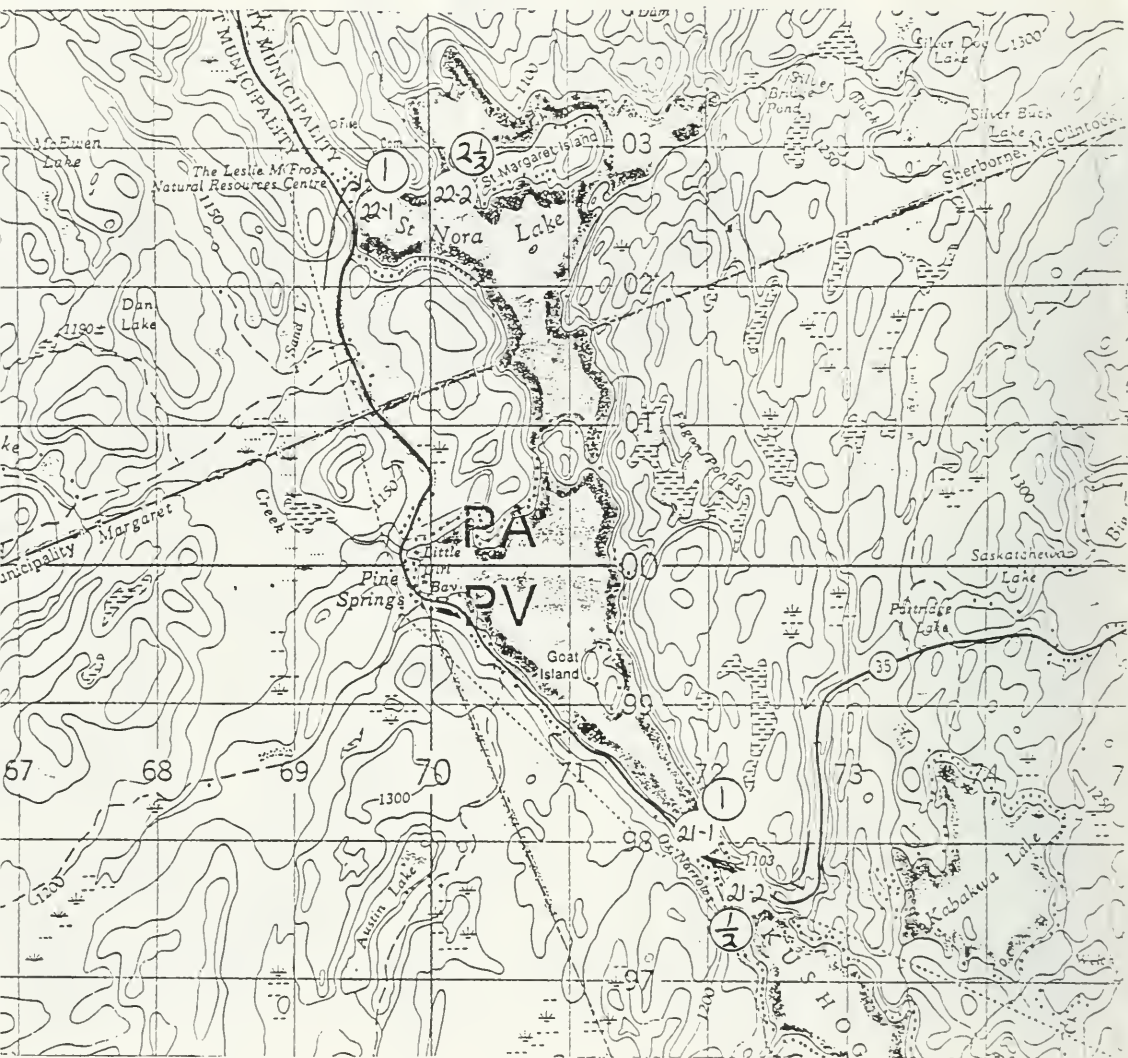
KUSHOG AND ST. NORA'S LAKE - July 5, 1987

Access: Kushog- divers only, either side of HWY 35 bridge.
        St. Nora - rear of Leslie Frost Research Station (sandy boat launch).

21-1, 21-2: Clam density greater above bridge, less below.
            Artificially introduced rock piles on bottom to aid fish spawning.

22-1: Anodonta and Eliptio observed.

22-2: Even distribution of Anodonta.

LAKE OF BAYS AND HARP LAKE - July 5, 1987

Boat access: (23-1) Launch and docks in town near motel area.
            (24-1, 24-2) off government docks.

23-1: Convenient diver access from docks.
      Continuous clam distribution above and below main Dorset bridge.

24-2: <u>Anodonta</u> and <u>Lampsilis</u> observed.
      No apparent <u>Eliptio</u>.
      Assorted macrophytes including <u>Isoetes</u>.
      Abundant wood detritus.

DEVELOPMENT OF A STANDARD CLAM BIOMONITORING METHODOLOGY

FOR THE DETECTION OF TRACE CONTAMINANTS

WITHIN WATERS OF

THE ONTARIO GREAT LAKES REGION

B - Evaluation of Alternative Field Methods

C - Determination of Temperature Effects

TABLE OF CONTENTS

EFFECTS OF TEMPERATURE AND FIELD PROCEDURES
ON PCB BIOACCUMULATION IN ELLIPTIO COMPLANATA.

Al Melkic and Yves Rollin, Integrated Explorations, P.O. Box 1385, Guelph,
Ont., N1H 6N8.

ABSTRACT: An experiment was designed to investigate the potential effects of
temperature and field handling methods on PCB bioaccumulation capacities of
the fresh water clam Elliptio complanata.

Clams subjected to various experimental regimes were simultaneously exposed
for 21 days to a common source of PCB contaminated water from the Niagara
River at Niagara-on-the-Lake. In-situ experiments were conducted in the river
to determine the effects of different deployment, transport, and processing
methods on PCB bioaccumulation. Laboratory experiments were conducted on shore
to determine temperature effects; water, held at constant temperatures of 5 -
25 degrees Celsius in environmental chambers, was pumped from the in-situ test
area of the river.

PCB levels were not found to be correlated with tissue or lipid weight. Lipid
weigh varied significantly between treatments in all but the processing
experiment.

No significant differences were observed in PCB levels between the various
transport treatments. Clams deployed in sand boxes contained the highest PCB
levels of all deployment treatments. However they also had the lowest lipid
levels. Processing technique did not have a significant effect on PCB
concentration nor did temperature. Acclimation of clams prior to deployment,
however, did increase PCB bioaccumulation by a factor of 2 - 6.

INTRODUCTION

The fresh water clam Elliptio complanata has been used by the Ontario Ministry of the Environment (OMOE) for the past 10 years as a standard in-situ, bioaccumulating agent to detect various trace contaminants in water. It's popularity stems from the fact that it is sedentary and thus represents the habitat in which it is found, as well as being a hardy species. The continued use of this popular technique has made it necessary to address a number of questions regarding the environmental factors which may limit it's practical application.

Over the past several years in Ontario, the OMOE and Environment Canada have conducted many biomonitoring studies using E. complanata (Kauss et al., 1981; Kauss, 1983). Integrated Explorations has been involved with many of these, including studies in the Niagara, St. Clair, and St. Mary's Rivers and in Port Hope Harbour. Due to our involvement, particularly with the implementation of these studies in the field, a number of questions have been brought into focus.

No standard method of transportation has been established to date. Typically, clams are maintained in Balsam Lake water at ambient temperatures or packed in ice. To ensure adequate oxygen levels, large amounts of water are used to transport the clams. Efforts have been made to deploy the clams within 24 hours of collection, however logistic problems have often caused variation in this period. With current deployment methods, normal clam behaviour is interfered with. Clams can not assume their normal syphoning attitude with their head down half buried in the substrate.

Processing methods to this point have been quite variable. Clams are usually shucked and frozen immediately upon retrieval (Innes et al., 1987), however, this is often not practical. At times clams have been kept on ice after shucking on site or kept alive on ice for varying periods after recovery before shucking and freezing (Hebert et al., 1985).

The temperature from which clams are taken is often quite different to that in which they are deployed, yet the effects of differences in temperature on bioaccumulation are not known nor are the effects of acclimation.

We were concerned with the effects of various treatments on the uptake of contaminants. An examination of data from previous clam bioaccumulation work, undertaken at Niagara-on-the-Lake (Niagara River Toxics Committee, 1984) indicated that PCB's were consistently present in concentrations above the detection limit. Therefore, it was chosen to serve as the model contaminant for our study.

The uptake of PCB's in E. complanata was used to investigate the effects of procedural and temperature variables on clam bioaccumulation capacity. The experiments were conducted simultaneously, during a single 21 day in-situ exposure experiment in the Niagara River at Niagara-on-the-Lake.

MATERIALS & METHODS

Field Methods

COLLECTION:

E. complanata specimens having a shell length of 6.5 to 7.2 cm were collected from Balsam lake, Rosedale, Ontario and transported to the experimental site at Niagara-on-the-Lake within 5 hours of collection, on October 21, 1986. All clams were transported in contaminant free, food grade plastic bags.

TRANSPORT EXPERIMENT:

Four transportation methods were investigated. Two methods involved transporting clams in large amounts of Balsam Lake water, one at Balsam Lake temperature of 11 oC (LTW) and the other at ambient temperature, which ranged from 15 to 20 oC (ATW). The two other treatments consisted of clams kept moist at either ambient temperature (ATM) or kept cool on ice (ITM). All clams were held for 48 hours before deployment in the Niagara River.

DEPLOYMENT EXPERIMENT:

Clams were deployed October 23, 1986 using five different deployment treatments (Fig. 1 & Fig. 2) on a platform (Fig. 2) located at approximately 3 metres depth. Three of the treatments, used modifications of the presently used clam cage, made of 1.5 cm diameter galvanized steel mesh. The floating cage (FC) suspended clams in individual pockets at mid depth, with all clams having the same orientation. The standard cage (SC) was similar to the floating cage but clams were oriented on their side instead of upright. Compact cages (CC) maintained clams jammed together at various orientations. Each of the caged treatments prevented clams from adjusting their orientation.

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Figure 1. Clam containment units used in deployment experiment
a) floating cage, b) standard cage, c) compact cage.

The other two treatments consisted of sand boxes and support rings. Sand boxes consisted of plastic trays filled with sand from Balsam Lake (SB) to simulate natural conditions. Each tray contained six 12.7 cm i.d. X 6.4 cm high ABS rings to prevent clams from congregating but still allowed clams to orient themselves. Support rings (SR) consisted of a series of rings, 6.4 cm high and 5 cm i.d. which held each clam in an upright position with syphons extended beyond the upper limit of the ring. Six clams were used in each treatment and maintained in the Niagara River for 21 days. Water temperature in the Niagara River ranged from 9 - 11 oC (11 $\pm$ 0.8, n=5 $\pm$ S.D.) during this time.

PROCESSING:

After a three week exposure period all clams were retrieved and processed as follows: Clams were shucked using clean hexane rinsed stainless steel knives; tissues were drained of excess fluid, wrapped in hexane rinsed aluminum foil and frozen on dry ice. Tissues were kept frozen at -11 C until analyzed.

Six treatments were investigated in the processing experiment: Three groups were held live on ice for 2 (LIH2), 8 (LIH8) and 24 (LIH24) hours before being processed; one group was shucked on retrieval and held on ice for 8 (SIH8) hours before being frozen; another group was held live at ambient temperature for 8 hours (LAH8) before being processed and the last group was processed immediately on retrieval (NH).
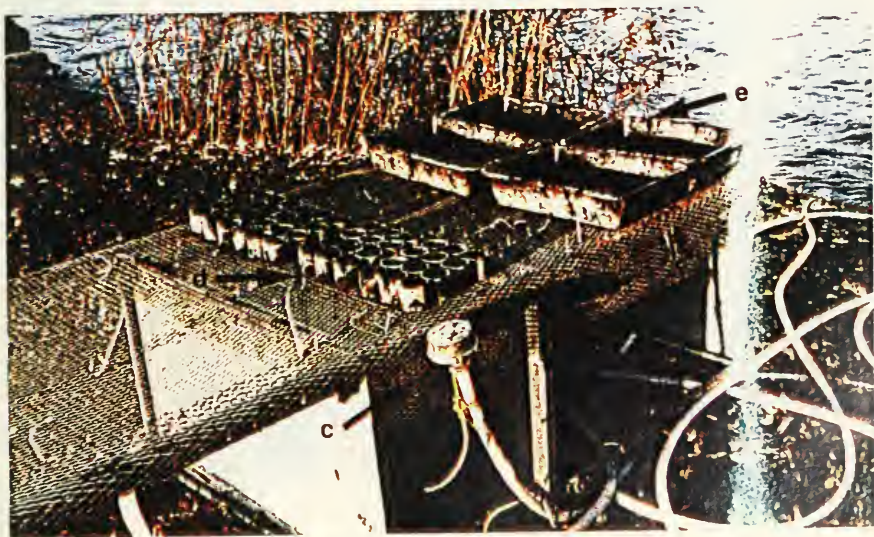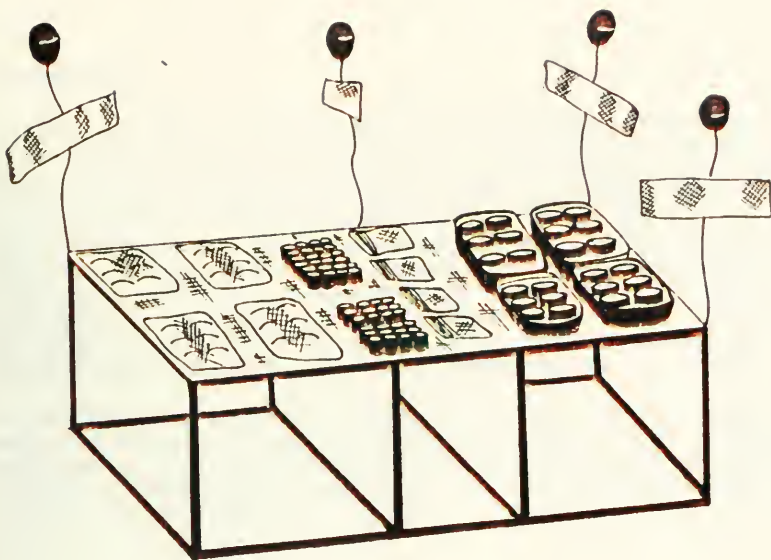
Figure 2. Rigid structure for mid-depth exposure of clams, to eliminate influence of vibration. Platform accommodated anchoring of clam containment units used in deployment experiment. a) Diagrammatic view of platform, b) Photograph of actual structure, c) Water intake for temperature experiment, d) Support rings, e) Sand boxes.

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TEMPERATURE:

An environmentally controlled system was established indoors (Fig. 3) in which 5 aquaria were maintained at 5, 10, 15, 20 and 25 oC and received a constant flow of fresh, thermally adjusted water from the Niagara River (Fig. 4). The source of water for this experiment was located on the underwater platform so that clams for all experiments were exposed to the same water. The thermal regimes were attained by mixing heated (26 oC) and chilled (4 oC) river water via a mixing manifold (Fig. 5). Temperatures within the individual aquaria were within 1 oC of the designated temperature throughout the duration of the experiment. A flow of 4 1/hr was maintained through the 12 1 aquaria (Fig. 6), which was sufficient to maintain both a high oxygen concentration and the desired thermal regime in the aquaria throughout the experiment.

In each aquarium, 6 thermally acclimated and 6 non-acclimated clams were supported upright in plastic rings (Fig. 6; same as SR in deployment experiment). The rings rested on a galvanized wire mesh to avoid sediment from being trapped within the rings. Temperature was monitored every 2 to 3 days for a 21 day period.
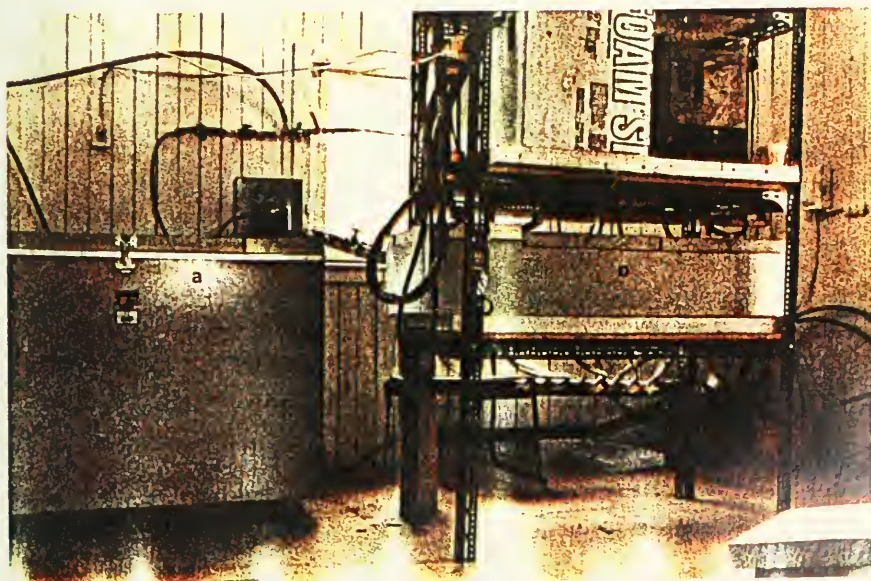
Figure 3. Environmental control system located at Niagara-on-the-Lake; a) cooling system, b) styrofoam box containing test aquaria.

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APPENDIX E. Raw data of the non-acclimated temperature experiment. Date refers to when samples were analyzed. Mean PCB concentrations are reported in ppb. PCB-t = ng PCB/g tissue, PCB-1 = ng PCB/g lipid.

Sample	Date (mm/dd/yy)	PCB-t	PCB-1	LIPID	WET WEIGHT (g)
5NA-1	05/03/88	80	85	0.94	4.99
5NA-2	05/03/88	31	29	1.07	6.08
5NA-3	05/03/88	8.0	8.1	0.98	5.37
		40	41	1.00	5.48
10NA-1	05/03/88	110	103	1.07	5.34
10NA-2	05/03/88	87	79	1.11	5.03
10NA-3	05/05/88	68	93	0.73	6.76
		88	91	0.97	5.71
15NA-1	05/04/88	75	63	1.18	5.91
15NA-2	05/04/88	4.6	4.7	0.98	6.79
15NA-3	05/04/88	40	34	1.17	5.79
		40	34	1.11	6.16
20NA-1	05/04/88	26	30	0.87	5.74
20NA 2	05/04/88	64	65	0.99	5.32
20NA-3	05/04/88	110	95	1.16	3.75
		67	63	1.01	4.93
25NA-1	05/05/88	78	110	0.71	5.76
25NA-2	05/05/88	49	126	0.39	5.15
25NA-3	05/05/88	130	73	1.78	3.91
		86	103	0.96	4.94

APPENDIX D. Raw data of the acclimated temperature experiment. Date refers to when samples were analyzed. Mean PCB concentrations are reported in ppb. PCB-t = ng PCB/g tissue, PCB-l = ng PCB/g lipid.

Sample	Date (mm/dd/yy)	PCB-t		PCB-1		LIPID		WET WEIGHT (g)	
5A-1	05/02/88	110		233		0.47		4.95	
5A-2	05/02/88	79		154		0.51		5.13	
5A-3	05/02/88	73	87	131	173	0.56	0.51	4.95	5.01
10A-1	05/02/88	83		144		0.57		5.58	
10A-2	05/02/88	173		247		0.70		5.51	
10A-3	05/02/88	160	139	269	220	0.60	0.62	4.84	5.31
15A-1	05/02/88	110		114		0.97		4.69	
15A-2	05/02/88	52		157		0.33		4.45	
15A-3	05/02/88	56	73	168	146	0.33	0.54	6.60	5.25
20A-1	05/02/88	34		40		0.85		4.49	
20A-2	05/02/88	169		459		0.37		5.01	
20A-3	05/02/88	67	90	163	221	0.41	0.54	4.42	4.64
25A-1	09/14/87	35		35		1.00		4.27	
25A-2	09/14/87	35		27		1.30		5.33	
25A-3	09/15/87	15	28	21	28	0.70	1.00	3.96	4.52

APPENDIX C. Raw data of the processing experiment. Date refers to when samples were analyzed. Mean PCB concentrations are reported in ppb. PCB-t = ng PCB/g tissue, PCB-1 = ng PCB/g lipid.

Sample	Date (mm/dd/yy)	PCB-t		PCB-1		LIPID		WET WEIGHT (g)
LIH2-1	09/21/87	23		15		1.50		4.18
LIH2-2	09/21/87	15		30		0.50		4.72
LIH2-3	04/04/88	62		83		0.75		6.56
			33		43		5.15	0.92
LIH8-1	09/21/87	7.5		11		0.68		7.46
LIH8-2	09/24/87	3.8		6.3		0.60		5.47
LIH8-3	04/04/88	49		64		0.76		5.06
			20		27		6.00	0.68
LIH24-1	09/21/87	9.0		30		0.30		6.03
LIH24-2	09/21/87	14		23		0.60		4.79
LIH24-3	04/04/88	50		35		1.43		5.37
			24		29		5.40	0.78
SIH8-1	09/14/87	41		41		1.00		6.56
SIH8-2	09/14/87	41		31		1.30		4.68
SIH8-3	09/15/87	35		27		1.30		5.99
			39		33		5.74	1.20
LAH8-1	09/14/87	27		30		0.90		5.82
LAH8-2	09/14/87	44		37		1.20		4.60
LAH8-3	09/14/87	24		27		0.90		4.14
			32		31		4.85	1.00
NH-1	11/13/87	8.3		11		0.78		5.48
NH-2	11/13/87	6.5		6.8		0.95		5.73
NH-3	03/29/88	40		46		0.87		6.34
			18		21		5.85	0.87

APPENDIX B. Raw data of the deployment experiment. Date refers to when samples were analyzed. Mean PCB concentrations are reported in ppb. PCB-t = ng PCB/g tissue, PCB-1 = ng PCB/g lipid.

Sample	Date (mm/dd/yy)	PCB-t	PCB-1	LIPID	WET WEIGHT (g)
LTWSR-1	11/13/87	8.3	11	0.78	5.48
LTWSR-2	11/13/87	6.5	6.8	0.95	5.73
LTWSR-3	03/29/88	40	46	0.87	6.34
		18	21	0.87	5.85
LTWCC-1	11/18/87	6.7	14	0.46	5.66
LTWCC-2	11/18/87	15	16	0.97	4.73
LTWCC-3	03/29/88	40	50	0.80	5.33
		21	27	0.74	5.24
LTWFC-1	11/18/87	31	43	0.72	7.39
LTWFC-2	11/18/87	19	23	0.82	5.61
LTWFC-3	11/18/87	66	97	0.68	5.12
		39	54	0.74	6.04
LTWSC-1	10/28/87	15	18	0.85	4.99
LTWSC-2	10/28/87	38	57	0.67	5.66
LTWSC-3	03/29/88	91	110	0.83	4.38
		48	61	0.78	5.01
LTWSB-1	03/21/88	32	148	0.22	5.80
LTWSB-2	03/21/88	21	108	0.19	6.81
LTWSB-3	03/21/88	74	236	0.31	5.57
		42	164	0.24	6.06

APPENDIX A. Raw data of the transport experiment. Date refers to when samples were analyzed. Mean PCB concentrations are reported in ppb. PCB-t = ng PCB/g tissue, PCB-l = ng PCB/g lipid.

Sample	Date (mm/dd/yy)	PCB-t	PCB-l	LIPID	WET WEIGHT (g)
ATMSR-1	09/24/87	5.3	8.8	0.60	6.21
ATMSR-2	09/25/87	6.5	9.3	0.70	6.00
ATMSR-3	03/29/88	50	106	0.47	5.59
		21	42	0.59	5.93
ATWSR-1	10/03/87	10	9.7	1.03	6.74
ATWSR-2	10/03/87	8.3	6.6	1.26	6.23
ATWSR-3	03/29/88	40	35	1.14	6.71
		19	17	1.14	6.56
ITMSR-1	11/13/87	18	20	0.90	7.12
ITMSR-2	11/13/87	6.3	6.3	1.01	5.09
ITMSR-3	11/13/87	7.7	8.8	0.88	4.86
		11	12	0.93	5.69
LTWSR-1	11/13/87	8.3	11	0.78	5.48
LTWSR-2	11/13/87	6.5	6.8	0.95	5.73
LTWSR-3	03/29/88	40	46	0.87	6.34
		18	21	0.87	5.85

APPENDICES

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CONCLUSION

1)   Under the conditions of this experiment, transportation method did not
      significantly affect PCB levels, indicating that clams can be
      transported in the field under broader conditions than has previously
      been assumed. However, since clams were stressed at ambient
      temperatures of only 15-20 oC, as indicated by slime production, and
      more extreme temperatures can easily be encountered, transportation of
      moist clams at ambient temperatures seems inappropriate.

2)   Deployment of clams in SB may be better than deployment by other methods
      due to apparently higher PCB levels. However further investigations
      aimed at reproducing these findings and elucidating why observed lipid
      levels were low is required before making definitive conclusions.

3)   Varying cage configurations do not appear to be a source of variation
      with relation to PCB levels enabling comparison of data from different
      studies.

4)   Clams were kept up to 24 hours on ice and up to 8 hours (submerged) at
      ambient temperature before processing commenced without affecting PCB
      levels. This may indicate that greater latitude can be taken with
      respect to how soon after retrieval clams have to be processed.

5)   Temperatures within the 5 - 25 oC range did not significantly affect PCB
      accumulation. This may increase the seasonal range of monitoring
      programs may be extended to include the colder months.

6)   PCB levels were consistently highest in clams at 10 C followed by clams
      20 C, in both the acclimated and non-acclimated treatments. There was
      also less scatter in PCB levels between temperatures in acclimated clams
      relative to non-acclimated clams.

7)   Clams acclimated prior to deployment accumulated significantly (2 - 6
      times) higher PCB levels than non-acclimated clams.

8)   Temperature and acclimation are not independent factors.

Table 9. Mean PCB (back transformed from log values on a lipid basis, ng PCB/g lipid) and lipid levels in clams of the temperature - acclimated experiment. Mean (n=3) PCB levels are reported with 95% confidence intervals while mean lipids are reported with their standard deviations.

TREATMENT (C)	PCB (ppb)	LIPID (%)
5	167 (80, 351)	0.51 + 0.026
10	212 (92, 491)	0.62 + 0.039
15	144 (86, 242)	0.54 + 0.21
20	144 (6.9, 3020)	0.54 + 0.15
25	27 (15, 50)	1.0 + 0.17

Table 10. Mean PCB (back transformed from log values on a lipid basis, ng PCB/g lipid) and lipid levels in clams of the temperature non-acclimated experiment. Means (n=3) PCB levels are reported with 95% confidence intervals while lipids are reported with their standard deviations.

TREATMENT (C)	PCB (ppb)	LIPID (%)
5	27 (1.5, 508)	1.0 + 0.038
10	91 (65, 126)	0.97 + 0.12
15	22 (0.49, 635)	1.11 + 0.065
20	57 (13, 242)	1.0 + 0.084
25	100 (50, 203)	0.96 + 0.42

Table 11. Summary of Duncan's New Multiple Range Test of average PCB levels (back transformed from log values on a lipid basis, ng PCB/g lipid) in clams of the temperature experiment. Treatment means underscored by the same line are not statistically different.

15NA	25A	5NA	20NA	10NA	25NA	15A	20A	5A	10A
22	27	27	57	91	100	144	144	167	212

Significant interactions were observed between temperature and acclimation as revealed by the analysis of variance. The two factors therefore do not affect PCB's independent.
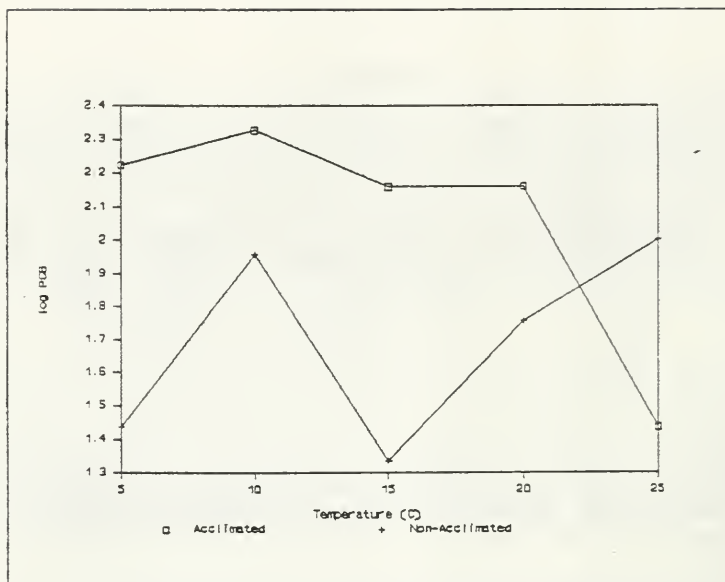
Figure 1. Effect of temperature on PCB concentration (ng PCB/g lipid) of acclimated and non-acclimated clams.
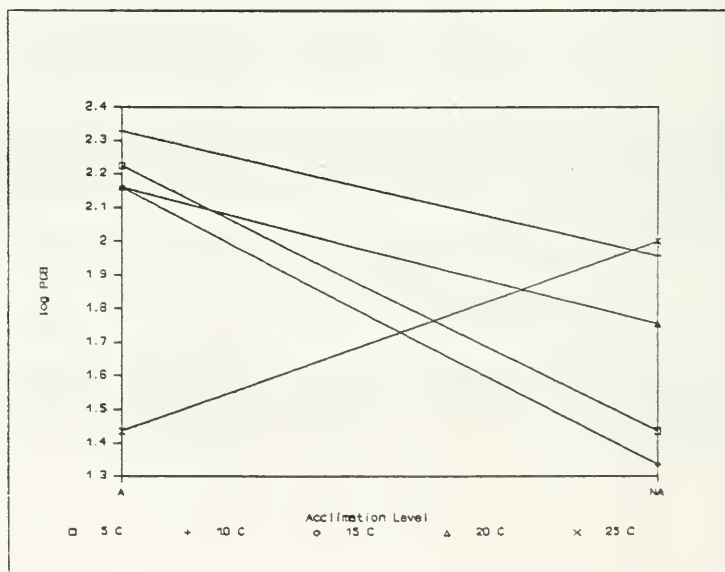

Figure 2. Effect of acclimation on PCB concentration (ng PCB/g lipid) at various experimental temperatures. A= acclimated, NA = non-acclimated.

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Temperature Experiment

Clams in the non-acclimated 15 C treatment had significantly (P<0.05) higher
lipid levels than in all other treatments, with the other treatments being
statistically similar (Table 8). Due to the difference in lipid levels PCB's
are reported on a per lipid basis (ng PCB/g lipid).

Table 8. Summary of Duncan's New Multiple Range Test of average lipid levels
(%) in clams of the temperature experiment. Treatment means underscored by
the same line are not statistically different.

5A	15A	20A	10A	25NA	10NA	25A	5NA	20NA	15NA
0.51	0.54	0.54	0.62	0.96	0.97	1.0	1.0	1.0	1.1

Further to being reported on a per lipid basis the PCB data was log
transformed prior to statistical evaluation to obtain normally distributed
data, since the original data was not normally distributed. All reported
means have been transformed back to non log units.

Temperature did not have a significant (P<0.05) effect on PCB accumulation in
either the acclimated (Table 9) or non-acclimated clams (Table 10).
Temperature did however appear to have an observable effect on clam activity
in both the acclimated and non-acclimated treatments (Fig. 1). Clams at 25 –
15 C showed active syphoning along with valve and foot movement, clams at 10 C
showed less active syphoning as well as less valve and foot movements, clams
at 5 C showed reduced siphoning activity relative to other temperatures, with
long periods of valve closures and no foot movement.

Significant differences (P<0.05) were observed in PCB levels between
acclimated and non-acclimated clams (Table 11). Acclimated clams had 2 – 6
times higher PCB levels than non-acclimated clams in all instances except at
25 C (Fig 2). These levels were significant (P<0.05) at 10 and 15 C. At 25 C
non-acclimated contained 4 times higher PCB levels than acclimated clams. No
explanation could be found for this.

Table 6. Summary of Duncan's New Multiple Range Test of average PCB (ng PCB/g lipid) levels in clams for the deployment experiment. Treatment means underscored by the same line are not statistically different.

LTWSR	LTWCC	LTWFC	LTWSC	LTWSB
21	27	54	62	164

Deployment of clams in SB may be better than deployment by other methods due to apparently higher PCB levels. However further investigations aimed at reproducing these findings and elucidating why observed lipid levels were low is required before making definitive conclusions.

Processing Experiment

Lipid levels were not significantly different ($P < 0.05$) between treatments and PCB data is therefore reported on a tissue basis (ng PCB/g tissue).

Levels of PCB's were not significantly different in any of the six processing methods utilized (Table 7.). Since PCB's are not readily lost during lipid metabolism (Stickel, 1973) nor are they volatile, these results are not unexpected. It would seem that the most practical method of processing clams is suitable for biomonitoring purposes. This would include holding the clams on ice for up to 24 hours or live at ambient temperatures for up to 8 hours before processing. Further experimentation may reveal that longer holding periods are possible, making biomonitoring more practical in remote locations. It must also be remembered that the ambient temperatures encountered in this experiment were moderate (15 - 20 oC) and that higher ambient temperatures could reduce the holding period.

Table 7. Mean PCB (ng PCB/g tissue) and lipid levels (%) in clams for the processing experiment. Means (n=3) are reported with their standard deviations.
LIH = live ice hold,   SIH = shucked ice hold,
LAH = live ambient hold,   NH = no hold
(2, 8, 24) = duration of the holding period, in hours.

TREATMENT	PCB (ppb)	LIPID (%)
SIH8	39 + 2.0	1.2 + 0.10
LIH2	33 + 15	0.92 + 0.30
LAH8	32 + 6.2	1.00 + 0.10
LIH24	24 + 13	0.78 + 0.34
LIH8	20 + 14	0.68 + 0.046
NH	18 + 11	0.87 + 0.049

Table 4. Summary of Duncan's New Multiple Range Test for average lipid content (%). Treatment means underscored by the same line are not statistically different.

LTWSB	LTWCC	LTWFC	LTWSC	LTWSR
0.24	0.74	0.74	0.78	0.87

PCB levels were significantly (P<0.05) higher in clams of the SB treatment relative to clams in the other treatments (Table 5 & 6.). Clams deployed in SR, CC, FC & SC were statistically similar to each other. PCB levels in LTWSB were approximately 3 - 8 times higher than in the other treatments. This may be due to a higher siphoning activity of clams in the SB due to a more natural orientation. Since PCB's are normally adsorbed to particles, bivalves must actively siphon to accumulate PCB's (Risebrough et al., 1976). Alternatively the high level observed may be an artifact caused by the low lipid levels in SB treated clams, since PCB levels on a per tissue basis (Appendix B.) were similar for all treatments in the deployment experiment.

Table 5. Mean PCB (ng PCB/g lipid) and lipid levels (%) in clams of the deployment experiment. Means (n=3) are reported with their standard deviations.
LTW = lake temperature wet,   SR = support rings,
CC = compact cage,   FC = floating cage,
SC = standard cage,   SB = sand box.

TREATMENT	PCB (ppb)	LIPID (%)
LTWSB	164 ± 38	0.24 ± 0.036
LTWSC	61 ± 27	0.78 ± 0.057
LTWFC	54 ± 22	0.74 ± 0.042
LTWCC	27 ± 12	0.74 ± 0.15
LTWSR	21 ± 13	0.87 ± 0.049

It is interesting to note that the clams in the SR treatment had the lowest PCB levels. This is unfortunate since SR was selected as our control because it seemed to present the most reproducible clam orientation. This may in part explain why low PCB levels were observed in the other experiments, which utilized the same control method of deployment.

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Experimental Findings

Transport Experiment

Clams from the ATMSR (transported moist at ambient temperature) treatment had significantly (P<0.05) lower lipid levels than clams from the other test groups (Table 2). The low lipid levels in the ATMSR group may be due to stress as indicated by the large amounts of slime excreted by the clams during transfer from Balsam Lake to the test site. Slime had been observed around the valves and siphons of the clams after being out of the water for 18 hours.

Since lipid levels were not similar for all treatments, PCB's are reported on a per lipid basis (ng PCB/g lipid). No significant differences (P<0.05) in PCB concentrations were observed in the four transportation methods tested. Mean PCB levels ranged from 12 ppb for clams transported at ice temperature moist to 42 ppb for clams transported at ambient temperature moist (Table 3).

Table 2. Summary of Duncan's New Multiple Range Test of average lipid levels (%) for the transport experiment. Means underscored by the same line are not statistically different.

ATMSR	LTWSR	ITMSR	ATWSR
0.59	0.87	0.93	1.14

Table 3. Mean PCB (ngPCB/g lipid) and lipid concentrations of clams in the transport experiment. Means (n=3) are reported with their standard deviations.
ATM = ambient temperature moist, ATW = ambient temperature wet, LTW = lake temperature wet,   SR = support rings.

TREATMENT	PCB (ppb)	LIPID (%)
ATMSR	42 + 32	0.59 + 0.066
LTWSR	21 + 13	0.87 + 0.89
ATWSR	17 + 9.0	1.14 + 0.066
ITMSR	12 + 4.2	0.93 + 0.040

Deployment Experiment

Lipid levels in the SB (sand box) treatment were significantly (P<0.05) lower than in other treatments, while other treatments were considered statistically similar (Table 4). PCB data was therefore reported on a per lipid basis (ng PCB/g lipid).

Statistical Analyses

A Chi squared "goodness of fit" test was used to determine if the data was normally distributed. Data which was not, was log transformed prior to statistical analysis and back transformed to present means.

Analysis of variance (ANOVA) was utilized to test for significant differences in the transport, deployment and processing experiments, with the individual experiments being treated as Complete Random Designs with equal replication. If the ANOVA revealed statistical differences, Duncan's New Multiple Range Test was used to elucidate treatment differences. The temperature experiment was analyzed as a 5 x 2 factorial experiment with temperature (five levels) and acclimation (2 levels) as the two factors. Paired comparisons were made between acclimated and non-acclimated clams exposed to the same temperatures using the least significant difference test.

All statistical analyses followed the procedures outlined in Steel & Torrie (1980).

RESULTS & DISCUSSION

General Findings

Mean lipid content in clam tissues was $0.82 \% \pm 0.039$ (mean of $n=75 \pm$ S.D.) with a range of $0.20 \% - 1.78 \%$. Kauss and Hamdy (1985) found similar lipid levels of 0.7 to 1.9 % in E. complanata. The average weight of clams was 5.51 ± 0.097 grams, with a range of $3.91 - 7.46$ grams.

PCB levels were not found to be correlated to lipid levels nor to clam weight. Correlation coefficients (r) for the regression of Y (PCB) on X (lipid) ranged from 0.024 to 0.507, and from 0.039 to 0.390 for the regression of Y (PCB) on X (weight). As a result, PCB levels were not normalized to a common lipid content or weight. However, PCB concentrations were reported on a per lipid basis (ng PCB/g lipid) in the transport, deployment and temperature experiments due to the significant difference ($P<0.05$) in lipid levels among clams within these experiments. Concentrations can be converted to a tissue basis (ng PCB/ g tissue) by multiplying by percent lipid.

The raw data for PCB concentrations, lipid levels and clam weights are presented in Appendices A to E.

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Laboratory Analysis

Clam tissue analysis of PCB's was done according to Ontario Ministry of the Environment protocol (OMOE 1983) with a few modifications.

Clam tissues were thawed, weighed and placed in 50 ml screw top , teflon lined centrifuge tubes with 20 ml of concentrated HCl and agitated for 1.5 - 2 hours. Extraction was performed using a 20 ml portion of 25 % dichloromethane in hexane (v/v) and agitated for a further 1.5 hours. Approximately 0.25 ml of 2-propanol was added to the centrifuge tubes to break the emulsion layer, then centrifuged for 20 minutes @ 2000 rpm. The extraction was repeated twice. Sodium bicarbonate, followed by sodium thiosulphate, was added to the extracts to neutralize and dry the samples respectively. Extracts were diluted to 100 ml with hexane and an aliquot, representing 1 g of tissue, used for cleanup with florisil 100-200 mesh in a dry packed column. The remainder of the extract was used for gravimetric lipid determination. Final PCB extracts were reduced to 1 ml using a rotary evaporator, made to 3 ml with 2,2,4-trimethylpentane (iso-octane) and submitted for analysis.

All PCB analyses were performed by gas chromatography using electron capture detection (GC/ECD). A Hewlett Packard 5880A Series GC, equipped with a 30 m x 0.32 mm I.D. SPB-35 capillary column (Supelco, Inc) was used. Typical GC conditions were: 80 oC isothermal for 0.50 minutes; then increased by 5 oC/minute to 300 oC, and held at 300 oC for 40 minutes. The splitless injection system (1.00 minute valve time) was maintained at 200 oC.

Levels of PCB's in the sample were determined by comparison to area response factors obtained for standard solutions of Arochlor 1260 and Arochlor 1254, injected separately.

Mean PCB recovery from controls (Appendix F.) was 88.4 % $\pm$ 4.4 % (mean of n=5 $\pm$ S.D.). Method detection limit was 8.9 ppb. No corrections were made for recovery efficiencies.

Table 1. Experimental design of procedure and temperature experiments. "c" indicates that the control conditions were used.

| PROTOCOLS | TREATMENT SCHEDULE | | | | Controls (c) |
	Transport Experiment	Deployment Experiment	Processing Experiment	Temperature Experiment	
Transport	trials	c	c	c	ambient temperature wet
Deployment	c	trials	c	c	ring support
Processing	c	c	trials	c	immediate processing
Temperature	c	c	c	trials	ambient river temperature

10
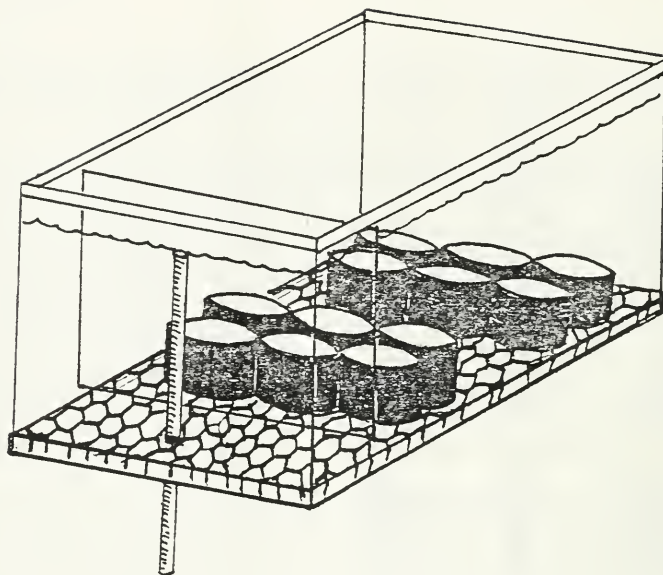
Figure 6. Diagram of aquaria used in the temperature experiment.

Clams obtained from Balsam Lake (10 C) were transported to Niagara-on-the-Lake in food grade contaminant free bags. On arrival at the test site, clams (still in bags) were placed in the environmentally controlled system at 10 C. Some of the clams remained at this temperature for 48 hours (non-acclimated), while others were acclimated over the same time period. The acclimation procedure consisted of removing bags of clams every 12 hours from an aquaria and placing them in aquaria 5 C warmer or colder. After 48 hours clams were simultaneously exposed to Niagara River water adjusted to the various temperatures

CONTROL:

In all experiments the control conditions consisted of clams transported in lake water at lake temperature (LTW), deployed in support rings (SR) and processed immediately (NH). These conditions were chosen as they were deemed to be the most reproducible. An overview of the experimental design is presented in Table 1.
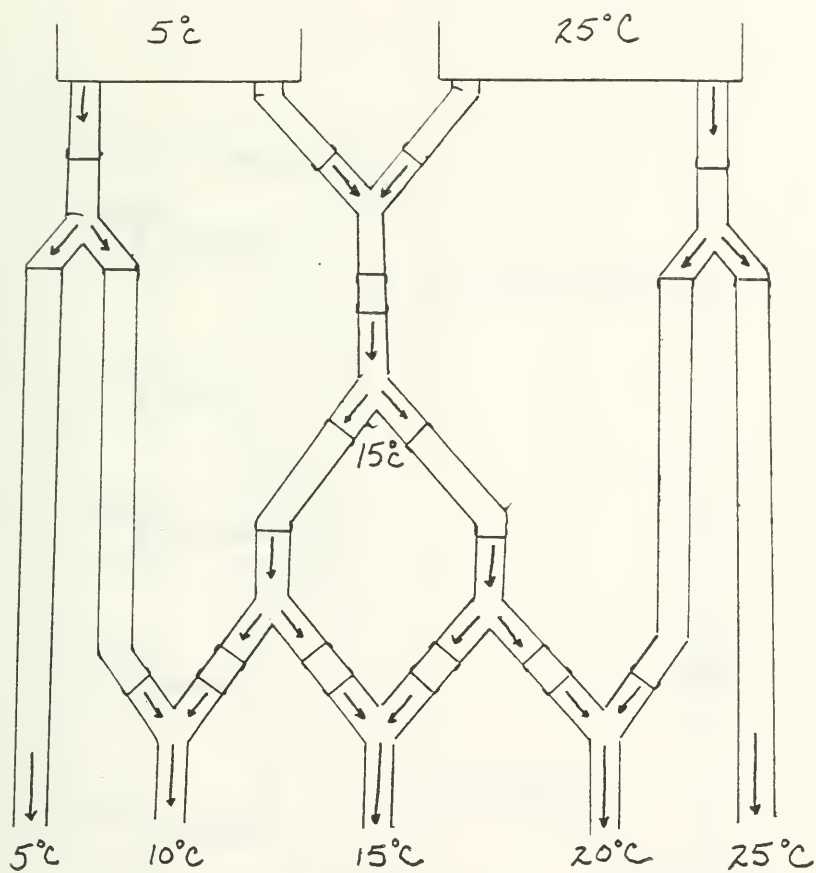
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Figure 5. Schematic diagram of mixing manifold used to achieve the various desired temperatures.
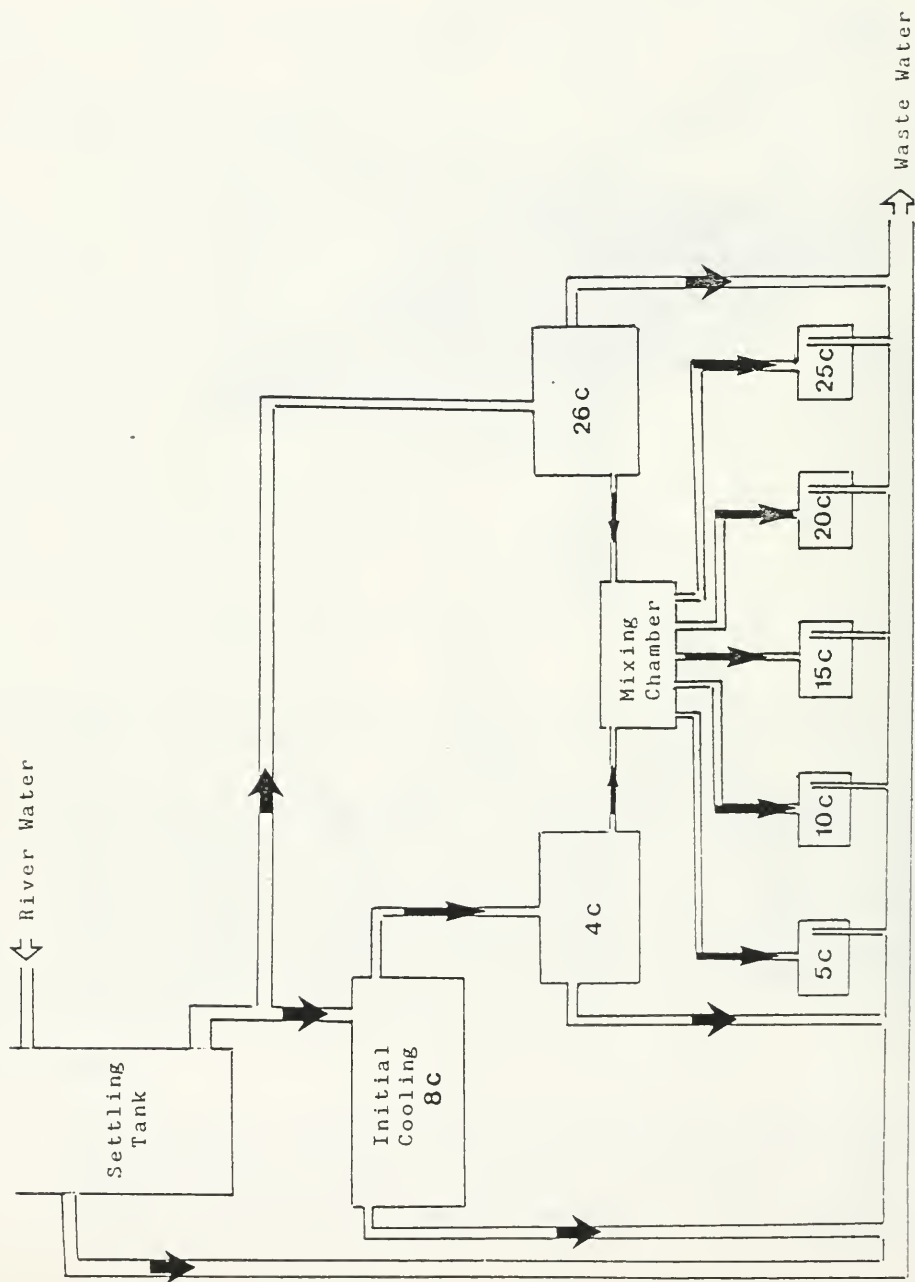
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Figure 4. Schematic diagram of the flow-through system used in the temperature experiment.